# DISEASE OUTCOME OF ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA BASED ON VIROLOGICAL RISK STRATIFICATION

**DEPARTMENT OF RADIOTHERAPY**

**CHRISTIAN MEDICAL COLLEGE**

**VELLORE 632004**

***DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF***

**MD BRANCH IX RADIOTHERAPY**

**EXAMINATION APRIL 2015**

**TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY**

**CHENNAI - 600032.**

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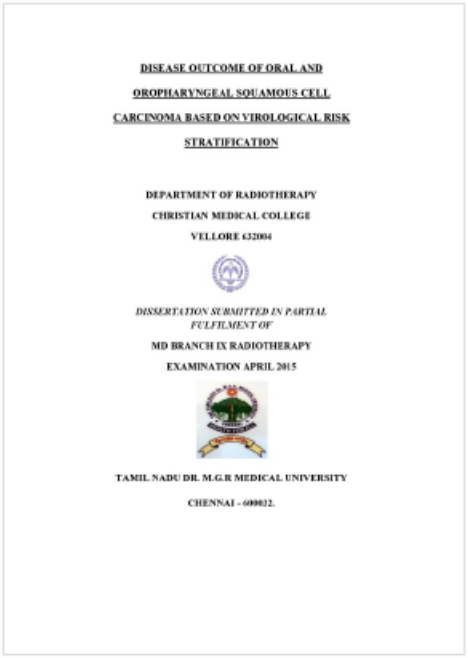

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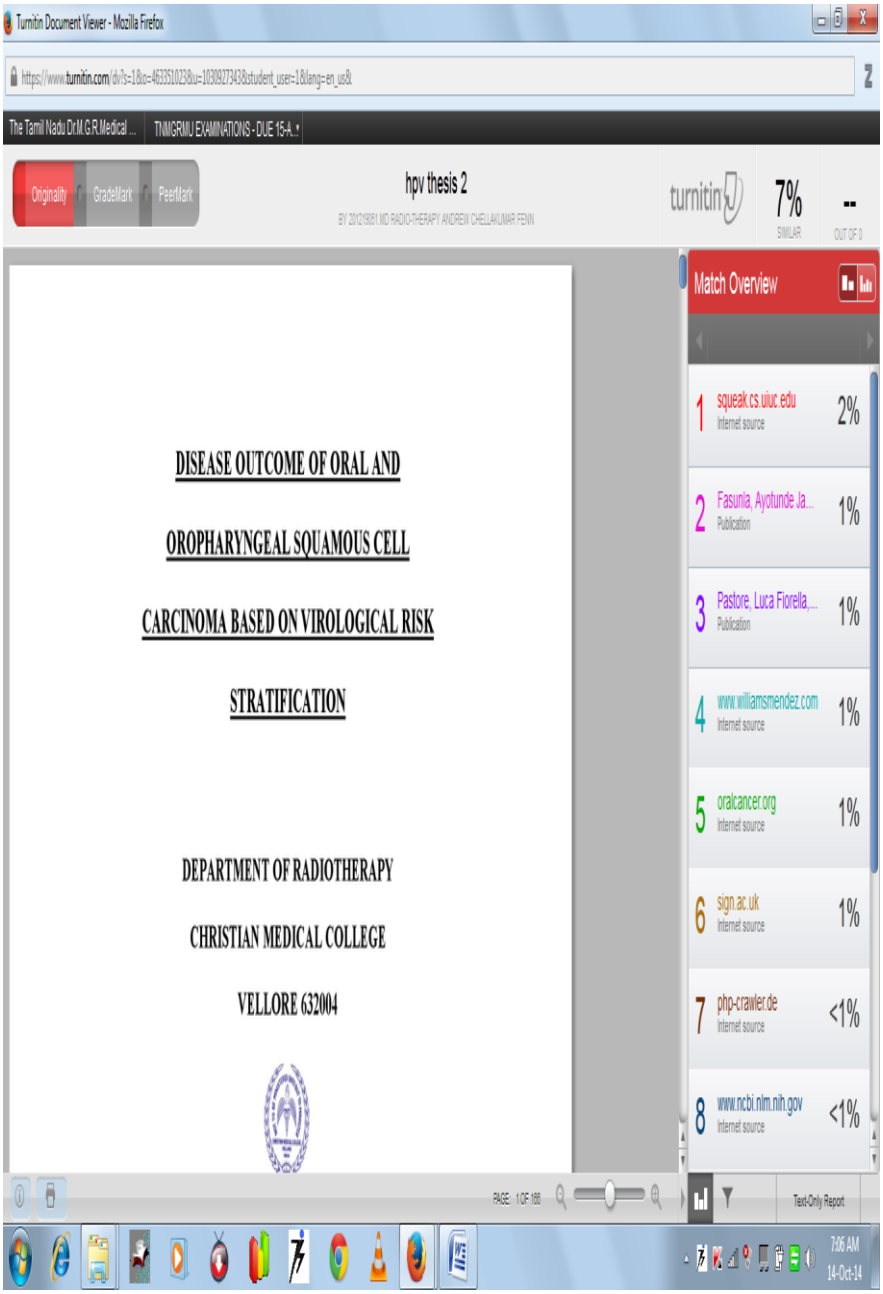
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Sub: **FLUID Research grant project NEW PROPOSAL**
Disease Outcome in Oral and Oropharyngeal Squamous Cell Cancers based on Combined Virological and Molecular Risk Stratification – A Cohort Study. Dr. Andrew C. Fenn, P.G. Registrar, Radiotherapy Unit – 2, Dr. Subhashini John, Dr. I. Rajesh, Dr. Saikat Das, Radiotherapy Unit 2, Dr. Priya Abraham, Clinical Virology, Dr. Meera Thomas, General Pathology, Dr. Rajinikanth, General Surgery I, Dr. Rajan Sundaresan, ENT unit I, Dr. Ramanathan, ENT unit II, Dr. Rajiv Michael, ENT III, Dr. Rabin Chacko, Dental and Oral Surgery Unit, Dr. Arun Paul S, Dr. Santhosh Koshy, Dental and Oral Surgery Unit- II

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Institutional Review Board

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Last, but not the least, I would like to thank my Lord and Saviour Jesus Christ, for giving me the strength and wisdom to complete this dissertation

# Contents

# ABSTRACT


## Study Title: Disease Outcome of Oral and Oropharyngeal Squamous Cell Carcinoma based on Virological Risk Stratification

### Introduction

Oral Cancer is the 9th most common cancer worldwide among men; it is the 6th most common cancer in the developing nations. Oral cancer ranks 2nd most common cancer among men in the South Central Asia region of which India is a part. In Chennai, head and neck cancers form about 20% of the cancer burden among men and a little less than 10% among women. The incidence rates of Oropharyngeal and Oral Cancers for men, of the total head and neck cancers, were 10% and 45% respectively. Traditional aetiology for head and neck cancers included tobacco and alcohol usage. But recently, infection with HPV was found to be an aetiological agent.

### Aims and Objectives

The aim of our study was to study the relationship of HPV infection to oral and oropharyngeal cancers. We wished to study the association between risk factors of HNSCC and HPV positivity, to evaluate the occurrence of premalignant lesions associated with HPV and to study the effect of HPV positivity on the aggressiveness of the disease and response to treatment.

## Methods and Materials

Based on the literature, we chose to divide the patients into 2 groups – HPV positive and negative. Sample size was calculated to be 18 in each group. All patients who were proven squamous cell carcinoma of the oral cavity and the oropharynx, treatment naïve and who did not have distant metastasis were included in the study. Patients with recurrence/metastasis were excluded from the study. Association of premalignant conditions of leukoplakia, erythroplakia and submucous fibrosis were looked at in relation to HPV infection. Various factors such as tobacco/alcohol use, HPV infection and response to therapy were looked at in oral/oropharyngeal cancers.

## Results

49 patients were available for HPV analysis, of which 2 patients' samples tested positive for HPV. There was no association found between HPV positivity and response to therapy. Also, both patients who were HPV positive had used tobacco. One patient who was HPV positive had associated Submucous Fibrosis.

## Conclusion

There is need to do more research in this field to clarify the role of HPV in Oral/Oropharyngeal Cancers in the Indian setting. Future studies looking at HPV would require having strict

inclusion criteria. Molecular profiling of these cancers based on p16 and p53 may further shed light on this emerging area of research.

## Introduction

Cancer is the most common cause of death worldwide, second only to heart disease.(1) Head and neck cancers form a good bulk of the cancer burden in developing nations. Whereas oral cancer is the 9$^{th}$ most common cancer worldwide among men, it is the 6$^{th}$ most common cancer in the developing nations.(1) Oral cancer ranks 2$^{nd}$ most common cancer among men in the south central Asia region of which India is a part.(1)

In India, particularly Chennai, head and neck cancers form about 20% of the cancer burden among men and a little less than 10% among women. The incidence rates of oropharyngeal and oral cancers for men, of the total head and neck cancers, were 10% and 45% respectively.(2)

In recent years, especially in the west, the incidence of head and neck cancers is on the decline due to decreased oral tobacco use.

But a trend of increased incidence of oropharyngeal cancers alone was noted.(3) On further investigation, this was found to be due to association with HPV (Human Papilloma Virus).(4)

In India, very few investigators have looked at the relationship between HPV and head and neck Cancers. Elango et al found a 48% prevalence of HPV among patients with cancer of the tongue.(5). Also in a study from the All India Institute of Medical Sciences, Delhi, 22% of patients with oropharyngeal cancers were found to HPV positive. They also made a notable observation that most of these patients were young and had multiple sexual partners which matched the patient cohort of HPV positive oropharyngeal cancers worldwide. (6)

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# EPIDEMIOLOGY

## Epidemiology

Tobacco and alcohol are important risk factors for the majority head and neck squamous cell cancers. Approximately 80 % of head and neck cancers are attributable to tobacco.(7) Smokers have an increased risk of over 10 times of getting head and neck cancer as compared to non smokers. (8) Heavy alcohol use is an independent risk factor for head and neck cancer.(9) Also alcohol and tobacco have a synergistic effect in causing head and neck cancer. (10).

As alluded to above, there was a decrease in the overall prevalence of head and neck cancers and an increase in the prevalence of cancers of the oropharynx, leading to the identification of a new etiological agent – Human Papilloma Virus (HPV).(4)

The prevalence of HPV related head and neck cancers is on the rise. There are varying rates of prevalence reported in literature. A systematic review published in the year 2005 reported HPV prevalence as 26% of all head and neck cancers worldwide.(11) Among these, Oropharyngeal Cancers had the highest prevalence (35%) followed in close succession by Laryngeal (24%) and Oral sites (23.5%).(11) In Asia, the prevalence rates were slightly

higher. Oropharynx accounted for 46% of these while Larynx and Oral Cavity accounted for 38% and 33% respectively.(11) Among the serotypes of HPV identified, 16 and 18 were the most common, whereas other oncogenic HPV viruses were rarely discovered. HPV 18 was most commonly identified in primaries outside the oropharynx.(11) In India, as already alluded to earlier, Bahl et al found the prevalence of HPV in oropharyngeal cancers to be 22%(6) and Elango et al found the prevalence of HPV in tongue cancers to be 48%.(5)

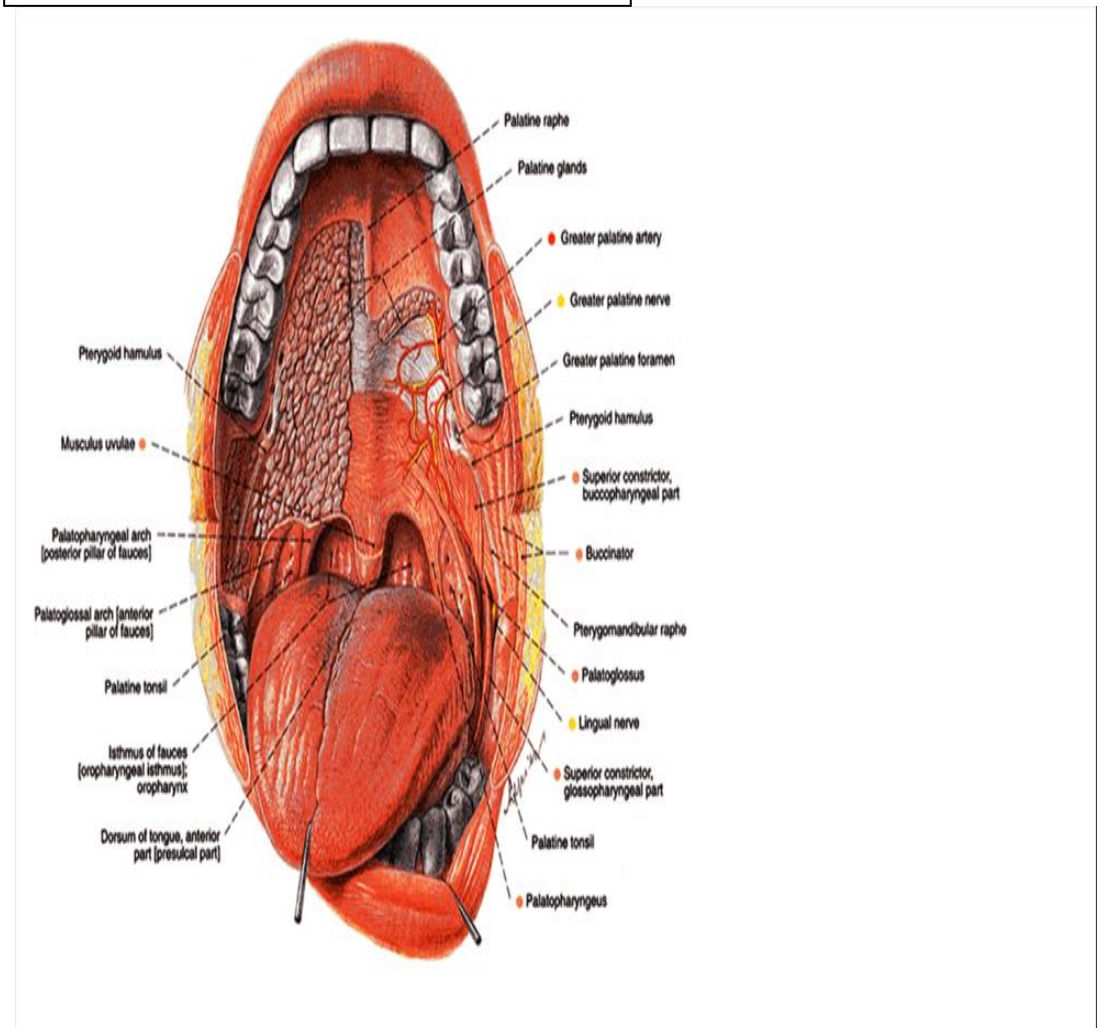
# ANATOMY

# Anatomy

The head and neck region has very complex anatomy. Basic consideration would be to divide the region into various sub divisions namely, Eye and Orbit, Nose and Paranasal sinuses, Nasopharynx, Oropharynx, Oral Cavity, Hypopharynx, Larynx and Salivary Glands. Some authors also include the Thyroid Gland in the anatomical description of the head and neck region.

## Oral Cavity

**Figure 1. Anatomy of Oral Cavity**




(12)

The oral cavity consists of the lips, oral tongue, floor of mouth, retromolar trigone, buccal mucosa, and hard palate.

The lips begin at the junction of the border with the skin and form the anterior aspect of the oral vestibule. They are defined into an upper and lower part. The motor control of the lips are supplied by the buccal and mandibular branches of the facial nerve.

The oral tongue is mobile and extends anteriorly from the circumvallate papillae to the undersurface of the tongue at the junction of the floor of mouth. The fibrous septum divides the tongue into right and left halves. The oral tongue can be demarcated into four anatomic areas: the tip, lateral borders, dorsal surface, and undersurface.

There are six pairs of muscles that form the oral tongue. Three of these muscles are extrinsic, while the other three are intrinsic. The extrinsic muscles are the genioglossus, hyoglossus, and styloglossus. The intrinsic muscles are the lingual, vertical, and transverse muscles. The extrinsic muscles primarily move the body of the tongue, while the intrinsic muscles alter the shape and conformation of the tongue during speech and swallowing.

The blood supply to the tongue is supplied via the lingual artery, tonsillar branch of the facial artery, and the ascending pharyngeal artery with primary drainage by the internal jugular vein.

General sensation of the anterior two thirds of the tongue is supplied by the lingual nerve. Taste fibers from the anterior two thirds of the tongue run in the chorda tympani branch of the facial nerve whereas the glossopharyngeal nerve provides sensation and taste to the posterior third of the tongue and circumvallate papillae.(13)

The floor of the mouth is a semilunar space extending from the lower alveolar ridge to the undersurface of the tongue. The floor of the mouth overlies the mylohyoid and hyoglossus muscles. The posterior boundary of the floor of the mouth is the base of the anterior tonsillar pillar. This region is divided into right and left by the frenulum of the tongue and contains the ostia of the submandibular and sublingual salivary glands.

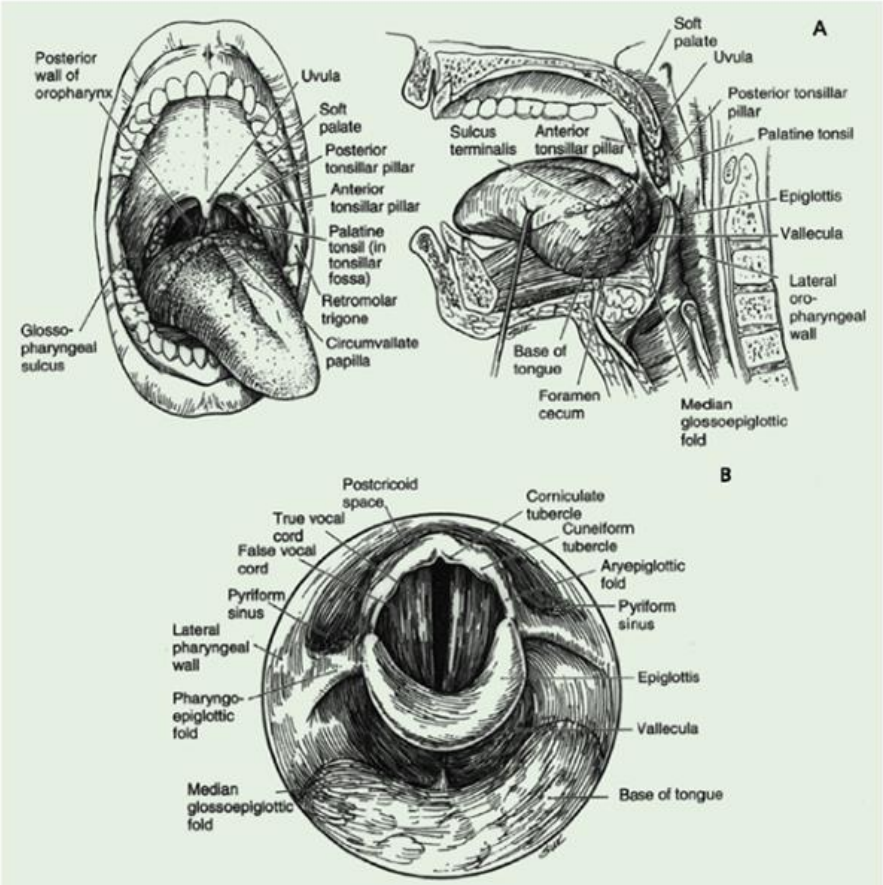
A sling formed by the mylohyoid muscles medially supports the anterior floor of the mouth, and the hyoglossus supports the posterior floor of the mouth.

The lingual and hypoglossal nerves are lateral to the hyoglossus, while the lingual artery is medial to the hyoglossus. Innervation of the floor of the mouth is provided by the lingual nerve.(13)
The retromolar trigone is a triangular area which overlies the ascending ramus of the mandible. The base of this triangle is formed by the posterior molar tooth and the apex lies at the maxillary tuberosity of the mandible.(13)

The buccal mucosa includes the mucosal surfaces of the cheek and lips from the line of contact of the opposing lips to the pterygomandibular raphe posteriorly. This extends to the line of attachment of the mucosa of the upper and lower alveolar ridge superiorly and inferiorly. Innervation is supplied by the buccal nerve, a branch of the mandibular nerve.(13)

## Oropharynx

**Figure 2. Anatomy of Oropharynx**




(14)

The oropharynx consists of various sub sites such as the posterior and lateral pharyngeal wall, tonsillar fossa, soft palate, and the base of tongue.(13)

The lateral walls of the oropharynx are limited posteriorly by the tonsillar fossa proper and the posterior tonsillar pillar. The anterior and posterior tonsillar pillars are the folds of mucous membrane that cover the underlying glossopalatine and pharyngopalatine muscles, respectively.

Deep to the lateral wall of the tonsillar fossa are major vessels and muscular components such as the superior constrictor muscle, the upper fibres of the middle constrictor muscle, the pharyngeus and stylopharyngeus muscles, and the glossopalatine and pharyngopalatine muscles. Stratified squamous epithelium covers all of these structures. Cranial nerves 9 and 10 supply these structures.(13)

The base of tongue lies posterior and inferior to the palatoglossal arch. It is bounded anteriorly by the circumvallate papillae, laterally by the glossopharyngeal sulci and oropharyngeal walls, and inferiorly by the valleculae and the pharyngoepiglottic fold.

Embryologically, its epithelium is derived from the endoderm, unlike that from the oral tongue (ectoderm).

The body of the base of tongue is formed by thick muscles, the genioglossus, styloglossus, palatoglossus, and hypoglossus muscles. The muscles originate from the margins of the mandible and are attached to the hyoid bone.

The blood supply and the innervation are by the lingual arteries and hypoglossal nerve, respectively.(13)

## Neck Nodal Levels

Level 1:

It starts from below the mylohyoid muscle to the lower margin of the hyoid bone and remains anterior to the posterior border of the submandibular glands

Level IA - submental nodes - between the anterior bellies of the digastric muscles

Level IB - submandibular nodes - posterolateral to the anterior belly of the digastric muscles

Level 2:

It starts from below the base of skull to above the inferior border of hyoid bone. It is anterior to the posterior border of sternocleidomastoid (SCM) muscle and posterior to the posterior border of the submandibular glands

Level 3:

It starts from the lower margin of hyoid to lower margin of cricoid cartilage, anterior to the posterior border of SCM.

Level 4:

It starts from the lower margin of cricoid cartilage to the level of the clavicle, anterior and medial to an oblique line drawn through the posterior edge of the sternocleidomastoid muscle and the posterolateral edge of the anterior scalene muscle

Level 5:

It starts from the base of skull up to the level of the clavicles posterior to the sternocleidomastoid muscles

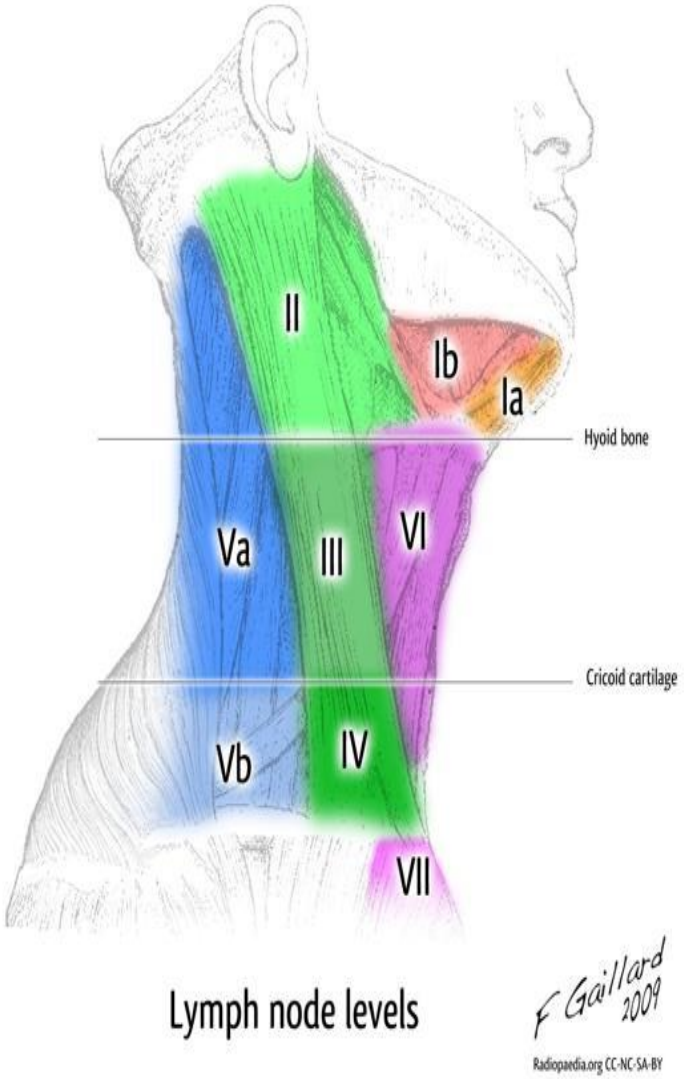
Level 6:

It extends from the inferior margin of the hyoid bone to the manubrium sterni, anterior to the levels 3 and 4.

Level 7:

It extends between the common carotid arteries, inferior to level 6 and deep to the manubrium

**Figure 3. Lymph Node Levels of the Neck**

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(15)

**Table 1. Incidence and distribution of regional metastasis for levels I-V for clinically N+ neck (in percentages)**

| Tumour site | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 |
|---|---|---|---|---|---|
| Oral | 48 | 39 | 31 | 15 | 4 |
| Oropharyngeal | 15 | 71 | 42 | 27 | 9 |

(16)

**Table 2. Incidence and distribution of regional metastasis for levels I-V for clinically N0 neck (in percentages)**

| Tumour site | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 |
|---|---|---|---|---|---|
| Oral | 20 | 17 | 9 | 3 | 0.5 |
| Oropharyngeal | 2 | 25 | 19 | 8 | 2 |

(16)

# PATHOLOGY

## **Pathology** (13)

The most common type of cancer in the oral cavity is squamous cell carcinoma. There are several variants of squamous cell carcinoma which include basaloid squamous cell carcinoma and verrucous squamous cell carcinoma. Basaloid squamous cell carcinoma is believed to have a worse prognosis than traditional squamous cell carcinoma. Verrucous carcinoma is generally considered a low-grade malignancy with low metastatic potential and good overall prognosis. (17) Sarcomatoid carcinomas can also be found in the oral cavity. These cancers have a poor prognosis with a mean overall survival of 2 years. (18) Less than 10% of neoplasms of the oral cavity have non squamous histology. Among these, tumours of the minor salivary glands are the commonest. Other histologies encountered are adenocarcinomas, melanoma, ameloblastoma, lymphoma, and Kaposi's sarcoma.

# STAGING

**TNM Staging Of Oral Cavity**

| | **T Stage** |
|---|---|
| **Tx** | Primary tumour cannot be assessed |
| **Tis** | Carcinoma in situ |
| **T0** | No evidence of primary tumour |
| **T1** | Tumour 2 cm or less in dimension |
| **T2** | Tumour more than 2 cm but not more than 4 cm in greatest dimension |
| **T3** | Tumour more than 4 cm in greatest dimension |
| **T4a** | Tumour invades local structures such as cortical bone, into deep muscles of tongue, maxillary sinus or skin of face |
| **T4b** | Tumour invades masticator space, pterygoid plates, or skull base and/or encases internal carotid artery |

| | **N Stage** |
|---|---|
| **Nx** | Regional lymph nodes cannot be assessed |
| **N0** | No regional lymph node metastasis |
| **N1** | Metastasis in a single ipsilateral lymph node 3 cm or less in greatest dimension |
| **N2** | |
| **N2a** | Metastasis in a single ipsilateral lymph node more than 3 cm but not greater than 6 cm in greatest dimension greatest dimension |
| **N2b** | Metastasis in multiple ipsilateral lymph nodes, none |

| | |
|---|---|
| | greater than 6 cm in greatest dimension |
| **N2c** | Metastasis in bilateral or contralateral lymph nodes, none greater than 6 cm in greatest dimension |
| **N3** | Metastasis in node greater than 6 cm |

| | **M Stage** |
|---|---|
| **M0** | No distant metastasis |
| **M1** | Distant metastasis is present |

| Anatomic Staging | T | N | M |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage 1 | T1 | N0 | M0 |
| Stage 2 | T2 | N0 | M0 |
| Stage 3 | T3<br>T1-T3 | N0<br>N1 | M0<br>M0 |
| Stage 4a | T4a<br>T1-T4a | N0-N1<br>N2 | M0<br>M0 |
| Stage 4b | Any T<br>T4b | N3<br>Any N | M0<br>M0 |
| Stage 4c | Any T | Any N | M1 |

(19)

**TNM Staging Of Oropharynx**

TX – Primary Tumour cannot be assessed

| | **T Stage** |
|---|---|
| **Tx** | Primary tumour cannot be assessed |
| **Tis** | Carcinoma in situ |
| **T0** | No evidence of primary tumour |
| **T1** | Tumour 2 cm or less in dimension |
| **T2** | Tumour more than 2 cm but not more than 4 cm in greatest dimension |
| **T3** | Tumour more than 4 cm in greatest dimension or extension to the lingual surface of epiglottis |
| **T4a** | Tumour invades the larynx, extrinsic muscles of tongue, medial pterygoid, hard palate or mandible |
| **T4b** | Tumour invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base or encases carotid artery |

| | **N Stage** |
|---|---|
| **Nx** | Regional lymph nodes cannot be assessed |
| **N0** | No Regional lymph node metastasis |
| **N1** | Metastasis in a single ipsilateral lymph node 3 cm or less in greatest dimension |
| **N2** | |
| **N2a** | Metastasis in a single ipsilateral lymph node more than 3 cm but not greater than 6 cm in greatest |

| | |
|---|---|
| | dimension greatest dimension |
| **N2b** | Metastasis in multiple ipsilateral lymph nodes, none greater than 6 cm in greatest dimension |
| **N2c** | Metastasis in bilateral or contralateral lymph nodes, none greater than 6 cm in greatest dimension |
| **N3** | Metastasis in node greater than 6 cm |

| | **M Stage** |
|---|---|
| **M0** | No distant metastasis |
| **M1** | Distant metastasis is present |

| Anatomic Staging | T | N | M |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage 1 | T1 | N0 | M0 |
| Stage 2 | T2 | N0 | M0 |
| Stage 3 | T3<br>T1-T3 | N0<br>N1 | M0<br>M0 |
| Stage 4a | T4a<br>T1-T4a | N0-N1<br>N2 | M0<br>M0 |
| Stage 4b | Any T<br>T4b | N3<br>Any N | M0<br>M0 |
| Stage 4c | Any T | Any N | M1 |

(19)

# NATURAL HISTORY

# Natural history

## Premalignant Conditions

| Table 3. Premalignant Conditions |
|---|
| Leukoplakia |
| Erythroplakia |
| Proliferative Verucous Leucoplakia (VRL) |
| Viadent Leukoplakia |
| Candida Leukoplakia |
| Reverse Smokings' Palate |
| Verrucous Hyperplasia |
| Oral Verrucous Carcinoma |
| Dyskeratosis Congenita |
| Actinic Cheilosis |
| Keratoacanthoma |
| Oral Submucous Fibrosis |

(20)

The above list details the number of premalignant conditions possible in the oral cavity. We will be dealing with only 3 of these, namely – leucoplakia, erythroplakia and oral submucous fibrosis.

## Leukoplakia

The WHO defines leukoplakia as a white patch or plaque that cannot be rubbed off or characterized clinically or pathologically as any other disease.(21) Leukoplakia is the most common precursor of cancer of the oral cavity. It is primarily a clinical entity with certain key pathologic features such as hyperkeratosis and acanthosis.  There are mainly 2 types – homogenous and non homogenous based on appearance. The non-homogenous type has a higher malignant potential

## Erythroplakia

The term erythroplakia describes a chronic, red, generally asymptomatic lesion or patch on the mucosal surface that cannot be attributed to a traumatic, vascular, or inflammatory cause. Erythroplakia, like leukoplakia, is a clinical diagnosis of exclusion that requires the clinician to rule out all other erythematous oral lesions.(22)

**Oral Submucous Fibrosis (SMF)**

Oral submucous fibrosis (SMF) is a chronic, progressive, scarring, precancerous condition which is characterized by mucosal roughness and rigidity. It has been causally associated with betel-nut chewing. SMF is more common in young adults, aged from 20−40 years. The most frequently involved sites in the oral cavity are the buccal mucosa, retromolar area, tongue and soft palate. Patients with SMF were at least 19 times more likely to develop squamous cell carcinoma compared to the normal population.(23)

## **Patterns of Spread** (13)

Cancers of the floor of the mouth can involve the ventral tongue and extend along the lingual nerve, submandibular duct, or invade the cortex of the mandible. They can invade deeply, involving the muscles of the floor of the mouth. There is an anatomical gap between the mylohyoid and hyoglossus muscles through which a cancer can gain access to submandibular and sublingual areas.

Carcinomas of the retromolar trigone tend to invade bone early.

Cancer of the buccal mucosa can invade the buccinator muscle and extend into the buccal pad of fat, and the subcutaneous tissue. The greater palatine foramina can allow tumours to spread posteriorly and enter the pterygopalatine fossa and skull base.

The hard palate has a dense mucoperiosteum and is relatively resistant to tumour invasion. However, the primary and secondary palates are fused at the incisive fossa and this is where tumours can gain access into the nasal cavity.

# MOLECULAR

# BASIS OF HPV

# INDUCED

# CARCINOGENESIS

## Molecular Basis of Human Papilloma Virus induced Carcinogenesis

Human papilloma virus (HPV) is more and more being identified as an aetiological agent in head and neck squamous cell carcinomas. Human papilloma virus is a non enveloped, double stranded DNA virus from the family papillomaviridae. (24) Although most HPV serotypes cause benign lesions such as anal warts or genital papillomas, a handful of them can cause cancer. As already alluded to earlier, the commonest of these are HPV 16 and 18.

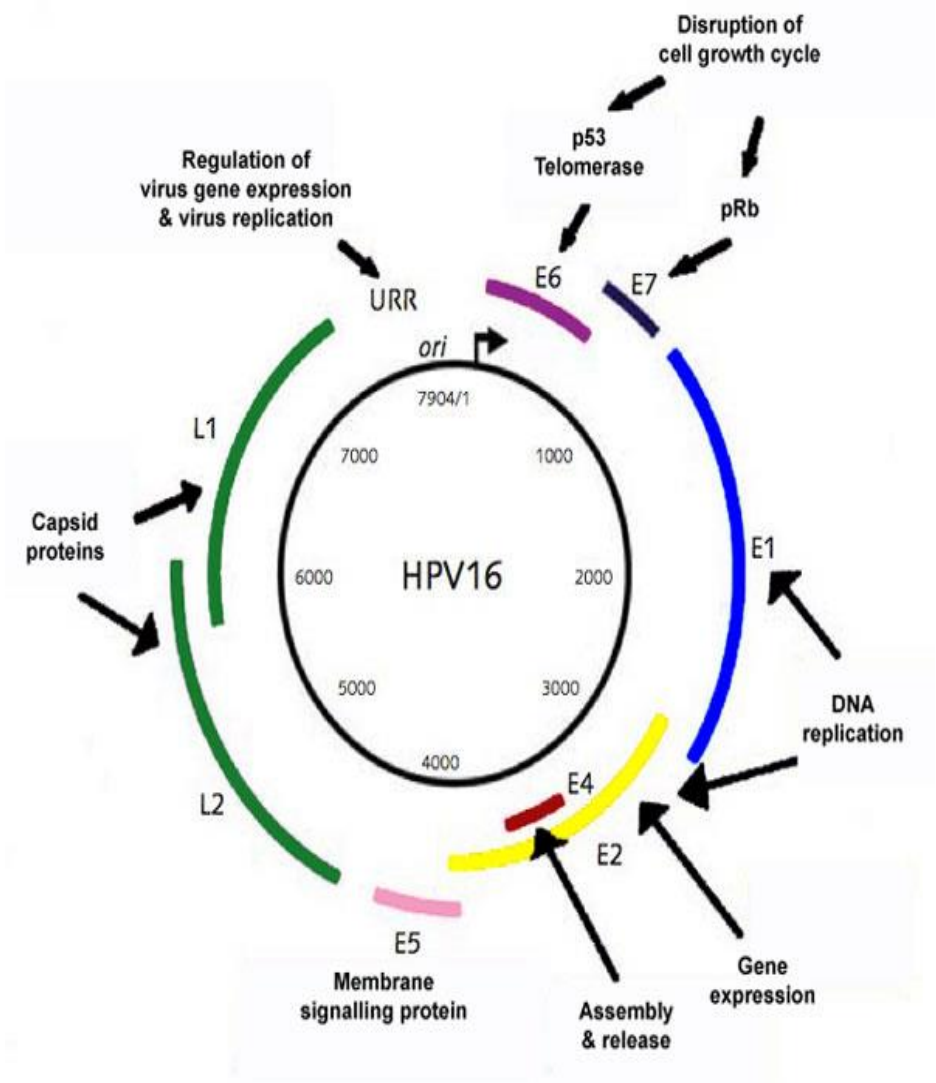
A very interesting fact about HPV cancers is it's relationship to p16, which is a cyclin dependant kinase. It is coded by the gene CDKN2A (Cyclin Dependant Kinase 2A). This gene is found to be up regulated in HPV related head and neck cancers.(25) p16 has been found to be a marker of good prognosis in HPV related Oropharyngeal Cancers (26–28), and has also been identified as a surrogate marker for HPV in cases of unknown primary with neck nodes (29) and in cervical high grade pre malignant lesions.(30,31)

The viral genomic machinery responsible for carcinogenesis is outlined as follows. On the Viral DNA are sections of the genome which are responsible for various actions as depicted in the figure 4.

E1-E7 are early open reading frames. They encode for non structural proteins responsible for various functions. E1 is responsible for episomal DNA replication. E2 is responsible for DNA replication and gene expression. It may also lead to repression of certain traits. E3 is a misnomer. E4 is responsible for assembly and release of viral proteins. E5 are membrane signalling proteins which help to relay information from inside the cell to the outside. The functions of E6 and E7 are enumerated below

L1 and L2 are late open reading frames and encode for structural proteins which are responsible for the viral coat, L1 being for the major capsid protein and L2 for the minor capsid protein

Figure 4. Map of the Human Papilloma Virus Genome with their respective functions




(32)

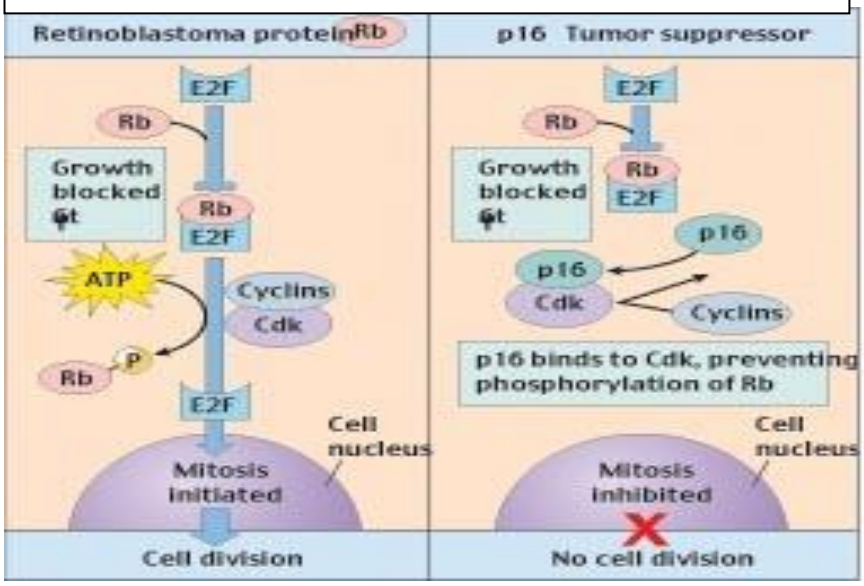
When an oncogenic HPV virus infects a squamous cell, it induces the formation of the viral E6 and E7 oncoproteins which lead to down regulation of the p53 and Rb (retinoblatoma) tumor suppressor genes respectively (Figure 5) and keeps them in a dormant state. (33). This releases the Eukaryotic Transcription factor 2 (E2F) to cause cell cycle progression.(34) (Figure 5)

p16 functions as a tumour suppressor by inhibiting cyclin D1 CDK4/CDK6 complex, preventing phosphorylation of retinoblastoma gene and negating the release of E2F resulting in cell cycle arrest. When Retinoblastoma gene is turned off by viral proteins, p16 activity is upregulated for unknown reasons. (Figure 2)

HPV positive/p16 positive head and neck cancers have been found to have a very favourable prognosis (35) and are the focus of several de intensification treatment regimens. (36)

It has recently been learnt that HPV analysis in recurrent or metastatic setting has prognostic significance supporting testing of the same in this group of patients.(37,38)

Fig. 5 Relationship between Retinoblastoma protein and p16




(39)

# DIAGNOSTIC

# WORK-UP

## Diagnostic work-up (13)

**Biopsy** of the lesion after a thorough work up needs to be performed

**Chest Radiograph** is taken to rule out metastasis

**Computed Tomography** (CT) scanning of the head and neck can provide accurate details on bony and soft tissue involvement as well as assessment of the neck nodes. It can help in determining the extent of invasion into the deep musculature of the tongue and adjacent structures. Moreover, CT is a valuable modality for visualizing invasion of the mandible, palate, and pterygopalatine fossa

**X-rays of the mandible** can also provide some assessment of bony involvement in the absence of CT scan.

**Magnetic Resonance Imaging** (MRI) of the face and neck is indicated when CT scanning will not help in assessment of local disease (for e.g., if a patient has a denture). MRI provides excellent definition of tumour involving the tongue and is a good modality for evaluating the possibility of perineural spread.

**Ultrasound Neck** can be used to determine the presence of enlarged neck nodes which are not clinically detectable. The accuracy of ultrasound neck when combined with FNAC (Fine Needle Aspiration Cytology) may be superior to MRI or CT scanning of the neck.(40)

**Positron Emission Tomography-Computed Tomography** (PET-CT) is a newer modality and has emerged as a one stop staging procedure. It has revolutionised the staging, treatment planning and follow up of head and neck cancer patients. Its strength is in evaluating persistent or recurrent disease, particularly in patients who have received previous radiotherapy.

## **Emerging testing modalities for HPV in HNSCC** (41)

**HPV16 E6 DNA Quantitative Polymerase Chain Reaction** (DNA qPCR):

Primer and a FAM-MGB labeled Taqman probe are harmonized to specifically amplify the HPV L1 region. The test uses fixed formalin paraffin embedded tissue.

**HPV16 E6 RNA Quantitative Polymerase Chain Reaction** (RNA qPCR):

Duplicate real-time RNA (complimentary DNA) PCR reactions are carried out using the Primer and a FAM-MGB labeled Taqman probe to look for the HPV16 E6 gene. The test uses fresh tissue.

**p16 Immunohistochemistry** (IHC):

p16 is scored as positive if the membrane staining is greater than 70%. All other staining patterns are termed negative. The test uses fixed formalin paraffin embedded tissue.

**High Risk HPV In Situ Hybridisation** (HR HPV ISH):

High risk HPV in situ hybridisation is carried out using proprietary reagents. It can detect various high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66).The test was scored as positive if there was any blue reaction product that co localised with the nuclei of malignant cells. The test uses fixed formalin paraffin embedded tissue.

The above tests have been evaluated in a prospective study by Schache et al.(41) The best combination of testing was to use HPV qDNA PCR along with p16 which gives a sensitivity and specificity of 97% and 94% respectively, compared with the gold standard of HPV RNA qPCR (Table 4). Other testing combinations did not give adequate sensitivity and specificity. Smeets et al. used p16 IHC as an initial screening test and followed it up with HPV DNA qPCR on the positive samples and found a sensitivity and specificity of 100%. (42)

**Table 4. Sensitiviy and Specificity of the Various Test/Test Combinations for HPV**

| | Sensitivity | Specificity | Statistical Significance |
|---|---|---|---|
| **HPV 16 status by RNA qPCR (Gold Standard)** | **(compared to RNA qPCR)** | | |
| **p16 IHC** | **0.94** | **0.82** | **<0.001** |
| **HR HPV ISH** | **0.88** | **0.88** | **0.001** |
| **Combined p16/HR HPV ISH** | **0.88** | **0.90** | **0.001** |
| **DNA qPCR** | **0.97** | **0.87** | **0.02** |
| **Combined p16/DNA qPCR** | **0.97** | **0.94** | **0.002** |
| **Combined p16/RNA qPCR** | **0.94** | **1** | **0.008** |
| **Combined DNA qPCR/RNA qPCR** | **0.94** | **1** | **0.001** |

(41)

# PROGNOSTIC FACTORS

## Prognostic factors

Lymph node positivity is the single most adverse prognostic factor for head and neck squamous cell carcinoma.(43)

Several pathological factors in the primary tumour are associated with poor prognosis.

Tumour thickness and depth of invasion have been shown to be associated with a higher risk of regional metastases. (17)

Perineural invasion has correlated with neck node metastases, extra capsular extension, and diminished survival. (44)

Vascular invasion has correlated with positive cervical lymph node metastases, (45) but not lymphatic invasion.(17)

The prognostic significance of grade has also been looked at and has not been found to an independent prognostic marker. (17)

**Table 5. Relationship between the occurrence of 5 year disease specific and overall survival in relation to the post operative nodal status of 266 oral squamous cell**

| | Disease Specific survival (5 year) Percentage | Overall survival (5year) Percentage |
|---|---|---|
| Pathologically N0 | 88 % | 75 % |
| Pathologically N+ | 65 % | 50 % |
| Pathologically N+ with ECE | 48 % | 38 % |

Disease Specific survival (5 year) Percentage
Overall survival (5year) Percentage
Pathologically N0 88 % 75 %
Pathologically N+ 65 % 50 %
Pathologically N+ with ECE 48 % 38 %

(46)

# PRINCIPLES

# OF SURGERY

## Principles of Surgery

Surgery is the mainstay of treatment in early stage oral cancer. It involves resection of the primary lesion, appropriate mandibular resection where necessary and reconstruction as needed.

Reconstruction may be in the form of primary closure, skin graft, regional flap, or free tissue transfer from different sites. These are decided based on the anticipated tissue loss, expertise of the surgeon and facilities available for the same.(47) An appropriate neck dissection is also performed. It can be a selective neck dissection, a Modified Radical Neck Dissection (MRND), Radical Neck Dissection (RND) or an extended neck dissection.

Standard radical neck dissection involves removal of the superficial and deep cervical fascia with its lymph nodes from levels I to V in continuity with the sternocleidomastoid muscle, omohyoid muscle, internal and external jugular veins, spinal accessory nerve, glossopharyngeal nerve and submandibular gland.

Modified radical neck dissection removes the superficial and deep cervical fascia with its enclosed lymph nodes and leaves one or more of the non lymphatic structures such as the sternocleidomastoid and digastric muscles, internal jugular vein, and spinal accessory nerve/glossopharyngeal nerve.

In selective neck dissection, one or more of lymph node groups I to V are not removed. There are various types – Supra omohyoid neck dissection and lateral neck dissection.

Supra omohyoid neck dissection removes the lymph nodes in levels I to III and is most commonly used for patients with small oral cavity cancers and a clinically node negative neck

The lateral neck dissection includes removal of level II to IV nodes and is often used for laryngeal, oropharyngeal, and hypopharyngeal cancers.

An extended radical neck dissection involves removal of additional lymph node groups or non lymphatic structures in addition to other structures removed while doing a radical neck dissection

# PRINCIPLES OF

# RADIOTHERAPY

## Principles of Radiotherapy

Radical radiotherapy is a viable option for the treatment of early oral and oropharyngeal cancers.

In case of oral cancers, some or all of the treatment needs to be administered with the help of brachytherapy to improve outcomes.(48) In a study done by Pernot, they reported local control rates of 96% for T1, 85% for T2, and 64% for T3 lesions of the oral cavity treated with brachytherapy/neck dissection. In the same series, local regional control rates were 83%, 70%, and 44%, respectively.(49) Advanced lesions require combined modality treatment – surgery and radiotherapy.

Although radiotherapy can be given as both neo adjuvant as well as post operative, post operative is preferred. (48,50). There are advantages with the post operative approach – no radiotherapy dose limitation, no delay in planned surgical resection, and complete pathologic staging of the tumour.

# PRINCIPLES OF CHEMOTHERAPY

## Principles of Chemotherapy

Concurrent chemotherapy has been the standard of care in locally advanced head and neck squamous cell cancers for the past decade. Several meta analysis (51,52) have shown the benefit of concurrent chemo irradiation. Although the designs of the trials included in the meta analysis were different, the basic comparison of radiotherapy in one arm with radiotherapy and concurrent chemotherapy in the other arm were made. The overall survival benefit quoted in the literature ranges from 1% - 8%. (53)

Neo adjuvant chemotherapy has not been the standard of care in oral cancers. A study done by Licitra et al found that overall survival was not changed with neo adjuvant chemotherapy but that the respectability and need for post op radiotherapy was reduced.(54)

The role of chemotherapy along with post op radiotherapy has been looked at. Two trials, one from Europe (EORTC) and another from USA have looked at this question. The inclusion criteria were slightly different. In general, they found that microscopically

positive margins, more than 2 nodes positive and extra nodal deposits, were associated with a better disease free survival if chemotherapy was added to radiotherapy. There was no benefit in overall survival in the American study while the EORTC trial showed both progression free survival as well as overall survival. (55,56)

Neo adjuvant chemo irradiation has not been adopted widely due to the prohibition of maximum radiotherapy dose to be administered. Also the chance of wound breakdown post surgery is a very likely possibility. A study done by Mohr et al. showed that pre operative chemo irradiation increased the overall survival and local control with the preoperative regimen but the regimen used was not very standard and was not convenient. They used 36 Gy at 2 Gray per fraction with concurrent Cisplatin 12.5 mg/m2 on the days of radiotherapy.(57)

# RADIOTHERAPY

# TECHNIQUES

## Radiotherapy Techniques

**Conventional Radiotherapy** uses parallel opposed lateral portals to cover the tumour and regional lymphatics. An anterior neck field is used in most cases to cover the pre tracheal nodes. After 40 Gy, spine shielding is done to limit the dose to the spinal cord. If required, the level 5 nodes are boosted up to 50 – 60 Gy with electrons, depending on the initial nodal burden. After 50 Gy, a shrinking field technique is applied and the primary and positive nodes are boosted to a maximum of 66 Gy.

**3D Conformal Radiotherapy** uses the same principles as conventional radiotherapy, but the field arrangements are different and dose to organs at risk are noted and kept to a minimum.

**Intensity Modulated Radiation Therapy** is a technique where the beams are modulated with the help of multileaf collimators as well as dynamic wedges, to produce a conformal dose distribution around the primary tumour and draining lymphatics with a margin. GTV (Gross Tumour Volume), CTV (Clinical Target Volume) and

PTV (Planning Target Volume) are contoured. The OAR's are contoured. The dose to each structure is determined and the plan is approved according to set constraints.

# DISEASE

# OUTCOME

## Disease outcome

In general, the disease outcome in HPV negative tumours is dismal.

Weinberger et al found that disease free survival and overall survival was improved in patients of oropharyngeal cancers with HPV and p16 positivity. (58) But this study also showed that HPV positive and p16 negative tumours had no difference in disease free survival and overall survival as compared to HPV negative tumours.

Another study done which looked at multiple factors affecting overall survival and disease free survival found that HPV positive tumours had improvement in both these parameters.(59)

A number of factors have been postulated to explain the improved survival with HPV positive tumours. These are improved radio sensitivity of these tumours,(60,61) immune surveillance of viral

antigens and absence of field cancerization because of most of these tumours are present in individuals who have never had tobacco or alcohol use.(62,63)

# AIM

## Aim

The aim of our study is to study the relationship of HPV infection to oral and oropharyngeal Cancers.

# OBJECTIVES

## Objectives

To study the association between risk factors of head and neck squamous cell cancers and HPV positivity

To evaluate the occurrence of premalignant lesions associated with HPV.

To study the effect of HPV positivity on the aggressiveness of the disease and response to treatment

# METHODS

# AND

# MATERIALS

## Methods and Materials

Patients were recruited using suitable inclusion and exclusion criteria from the OPD's of the departments of Radiotherapy, ENT, General Surgery Unit 1 and Dental Surgery.

### Inclusion Criteria

**Pathological**: Patients with pathologically proven squamous cell carcinoma of the oral Cavity and oropharynx of age above 18 years (adults)

**Clinical Stage**: Any T, Any N without distant metastasis or recurrence as determined by **clinical evaluation** [Includes History, Physical examination and Nasopharyngolaryngoscopy{NPL Scopy}/Direct LaryngoScopy {DL Scopy}] and Imaging.

### Exclusion Criteria:

1. Metastatic or recurrent Disease.
2. Adequate tumour tissue not available for assessment due to prior biopsy/excision at an outside centre.
3. Any prior therapy received for the malignancy for which the patient is presenting at present.

4. Pregnant women.

Metastatic or recurrent cancers were excluded because of data stating that patients with HPV positive cancers in this cohort of patients had similar outcome to HPV negative patients. (64)

## Procedure

Once a potential patient was identified by clinical and/or imaging features or with an outside biopsy of squamous cell cancer, he/she along with the relatives are counselled regarding inclusion in the study and informed consent is obtained. Once informed consent was obtained, biopsy of the suspected lesion was done in the Department of Dental and Oral Surgery (Oral Malignancy) or Department of ENT (Oropharyngeal Malignancy).

At the time of biopsy, 2 containers were required:

1. Standard formalin histopathology specimen which was sent to General Pathology Lab
2. Small cold chain specimen container with reagent for HPV analysis (25mg, 0.4x0.4 cm size) which was sent to Clinical Virology Lab.

If the biopsy was conclusive of squamous cell carcinoma, staging work up was done. If biopsy was inconclusive, a second biopsy was done. If the second biopsy was also inconclusive or negative for squamous cell carcinoma and/or the staging workup was positive for metastasis, then the patient was excluded from the study. Once all work up, dental clearance and cardiology clearance was completed, patient proceeded for treatment based on the site and stage of disease.

Following completion of treatment, patients were followed up every 3 months for a year.

## Assessment Variables

The presence of the various risk factors for HNSCC were recorded

1. Smoking
2. Pan Chewing (smokeless tobacco)
3. Alcohol

The history of sexually transmitted disease was recorded

The presence of the following pre malignant lesions were recorded.

1. Leukoplakia
2. Erythroplakia
3. Submucous Fibrosis

The presence of residual/local recurrence, regional and distant metastasis was looked at in relation to HPV negative and HPV positive patients.

## Treatment

**Radical Surgery (Oral Cancers)** included a wide excision of the primary tumour + appropriate reconstruction and ipsilateral supra omohyoid node dissection (level 1-3) in clinically node negative disease and radical/modified radical neck dissection (level 1-5) when nodes were clinically palpable.

**Radiotherapy in the Post Operative setting** was given if the following conditions were fulfilled

1. Large primary - T4 or T3 with soft tissue infiltration
2. Close margins of excision
3. Deep infiltrative tumour
4. Lympho-vascular and perineural invasion
5. Bulky nodal disease N2 / N3

Indications for addition of chemotherapy to radiotherapy was

1. Presence of extranodal deposits
2. Positive surgical margins.

**Radiation doses to be used for Radical and Post Op settings** was given 60 – 66 Gray at 2 Gray per fraction to the Primary disease (5 – 6 days a week) and 46 – 50 Gray for the prophylactic lymph nodal area (5 - 6 days a week).

**Concurrent chemotherapy** was planned as follows:

Inj. Cisplatin was administered (40mg/m2) once weekly for a maximum of 4 cycles.

Or

Inj. Cisplatin was administered (100mg/m2) once every 3 weeks for a total of 2 – 3 cycles based on tolerance.

**Palliative Radiotherapy** was executed with radiation doses of 40 Gray in 10 fractions to the primary and draining lymph node areas at highest risk of disease (2 fractions a week).

Patients were reviewed weekly during Radiotherapy for potential reactions or more frequently if required. They were also advised to use regular mouth washes and vitamins during the period of radiotherapy and for 4 – 6 weeks afterward. If necessary, Ryle's tube feeding and nutritional advice were initiated if deemed appropriate by the treating physician.

**HPV status by DNA PCR and RNA PCR**

These methods are described in detail as follows:

***Sample collection*:**

- Biopsy tissue in viral transport medium was collected and transported in an ice container.
- Viral transport medium: Balanced isotonic solution at physiological pH. It maintains the virus in the viable state. Generally contains fetal calf serum and antibiotics.
- Once received, the sample is transferred to a 1.5ml eppendorf tube and stored at $-80^0$C until further testing.

***DNA Extraction protocol***

DNeasy® Tissue kit: (Qiagen GmbH, Hilden, Germany)

Principle: Column based separation.

- Tissue is cut into small pieces weighing 25mg and it is digested by adding ATL buffer and Proteinase K at 56C.
- Once digested, an equal amount of AL (Lysis buffer) buffer and Ethanol is added.

- The DNA gets precipitated and its washed twice by adding buffers (AW1 and AW2)
- The DNA is then eluted by adding elution buffer.
- The extract (containing the DNA) is stored at -20C until further testing.

***PCR***:

- A known positive control is used for PCR and beta-globin serves as internal control.
- Primers used: a) PGMY 09/11: Target size: 450 base pair.
  b) PCO4/GH20 (Beta-globin): 230 base pair
- A sample can be analyzed only if the beta globin is positive.
- Based on the presence or absence of target band (450bp), the sample is interpreted as positive or negative.

***Sequencing:***

- If sample is positive for HPV, the amplified PCR products will be purified by Millipore filtration and sequenced directly using an ABI Prism Big Dye terminator cycle sequencing ready reaction kit.

- After a post-sequencing clean-up by Millipore filtration, the sequencing reactions will be run on an ABI PRISM 310 genetic analyzer (PE Applied Biosystems, CA, USA).
- Finally, the data will be analyzed using Bioedit software version 7.0.5.3 and study sequences compared to the GenBank HPV sequences.

***RNA Extraction and Amplification by Real Time PCR***:

Studies for mRNA will be done by Ambion RNA extraction protocol. Principle is column based separation. E6 and E7 mRNA oncogene expression reflects a direct viral involvement in the carcinogenesis. Amplification of HPV 16 and 18 will be done using real time PCR based on Taqman chemistry. Primers and probes will target the E6/E7 region of HPV 16 genome (80-120 base pairs). Similarly, the E7 region of HPV 18 will be chosen for quantitation.

***Isolation of Genomic DNA:***

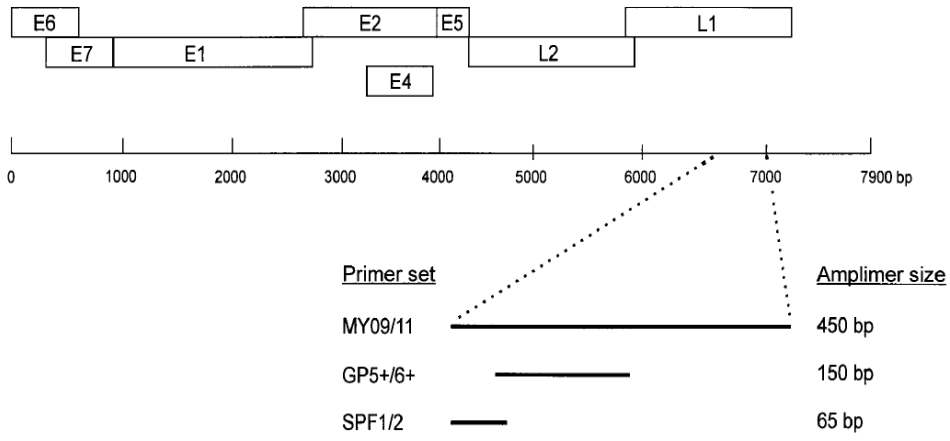
Fresh tissue collected at the various OPD's will be transported in a viral transport medium. DNA will be extracted using the DNeasy®

Blood and tissue kit (Qiagen GmbH, Hilden, Germany). The extraction protocol followed as per the manufacturer instructions.

***HPV PCR***:

HPV L1 region will be amplified using PGMY 09/11 primers. PC04/GH2O was used as the internal control (Housekeeping gene) to check for the integrity of the sample.

**Figure 6. Schematic representation of different primer sets for HPV DNA detection. Primers targeting the L1 gene of HPV are the most widely used.**



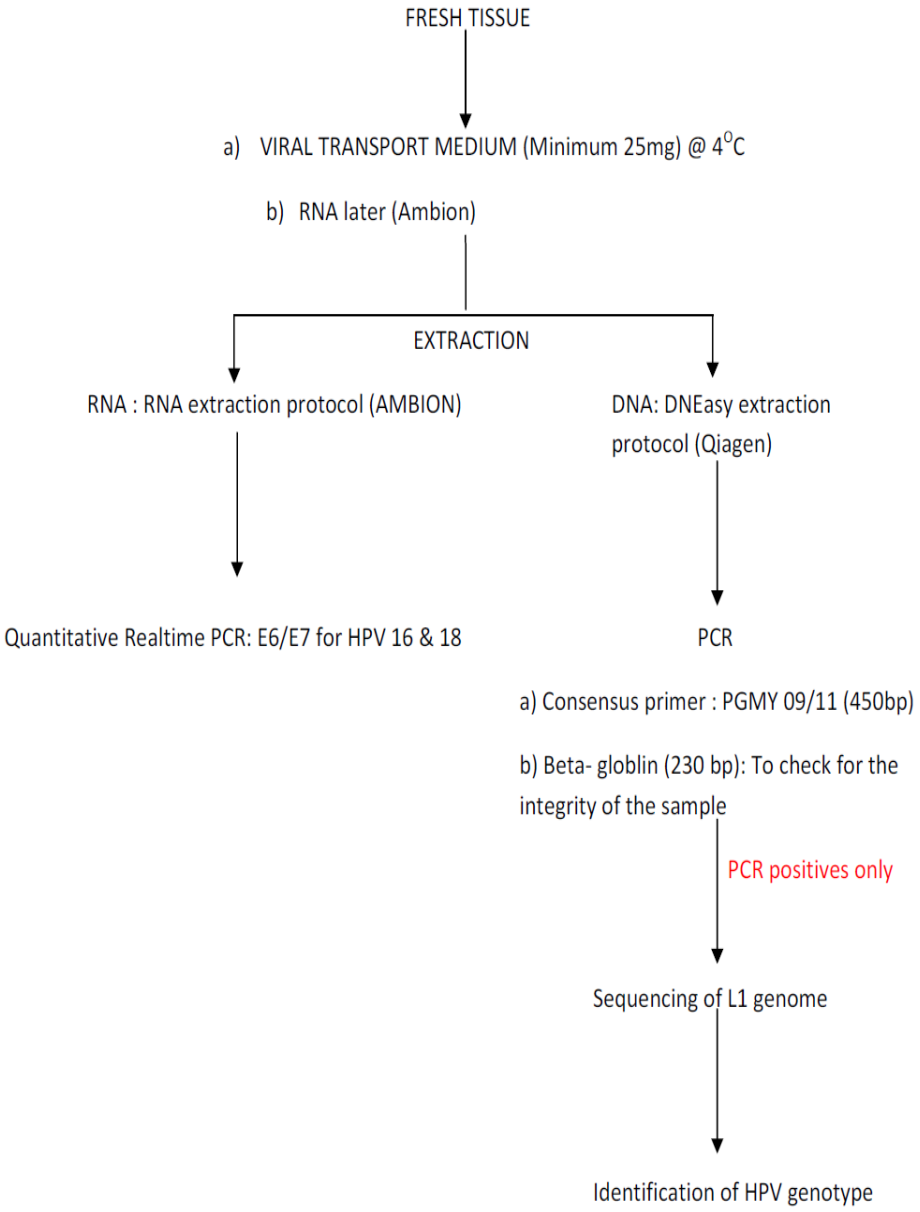

(65)

The PCR will be carried out as a single step non-nested PCR. These universally used primers would detect most of the oncogenic HPV types including HPV 16 and 18. Detection of the amplified products will be carried out by electrophoresis in an ethidium bromide stained 2.5 % agarose gel. Presence of a 450 base pair HPV DNA specific product and a 230 base pair beta globin specific product indicates a positive result. PGMY09/11 is a set of consensus primers which has higher sensitivity, specificity and reproducibility. (66)

The cycling conditions in the PCR are 95$^{o}$C, 10min followed by 40 cycles of 95$^{o}$C, 1min; 55$^{o}$C, 1min and 72$^{o}$C, 10min and a final elongation step of 72$^{o}$C, 10min.

The thermal cyclers to be used for PCR are

a) Veriti TM Thermal Cycler (Applied Biosystem, Foster City, California, USA).
b) GeneAmp® PCR system 9700 (Applied Biosystem, Foster City, California, USA).


FRESH TISSUE
a) VIRAL TRANSPORT MEDIUM (Minimum 25mg) @ 4°C
b) RNA later (Ambion)
EXTRACTION
RNA : RNA extraction protocol (AMBION)
DNA: DNEasy extraction protocol (Qiagen)
Quantitative Realtime PCR: E6/E7 for HPV 16 & 18
PCR
a) Consensus primer : PGMY 09/11 (450bp)
b) Beta- globlin (230 bp): To check for the integrity of the sample
PCR positives only
Sequencing of L1 genome
Identification of HPV genotype

## Sample size

Class I: HPV negative, p16 low; 5 yr local recurrence: 45%

Class II: HPV positive, p16 low; 5 yr local recurrence: 74%

Class III: HPV positive, p16 high; 5 yr local recurrence: 14%

Sample size calculation: (outcome vs. local control at 5 yrs)

These values were obtained from the study done by Weinberger et al. (58) The local recurrence rates of classes I and II were clubbed and compared to the recurrence rate of class III as class III represented a molecular and virological true model of HPV. Class II, even though being HPV positive, had a worse outcome than the other 2 groups and was more similar to the HPV negative group. Hence, it was clubbed together with it for sample size analysis. There is data to support that Tobacco and alcohol usage may downplay the beneficial effects of being HPV positive and hence a distinction may be seen.(67)

The following formula was used for calculation of sample size

$N= 2\ p'q'\ (z_\alpha + z_\beta)^2 / (p1-p2)^2$

p1= recurrence rate of class III = 14% (approx)

p2= recurrence rate of class II and I = 60% (approximately 74+45/2)

p'= (p1+p2)/2 = 37

q'= 100-p' = 63

$z_\alpha$ = type I error = 1.96 at 5% level

$Z_\beta$ = type II error = 0.84 for β=20%

This gives a value of 17.27 (approx. 18)

So sample size would be 18 in each group.

## Analysis

The data was entered in Epi Data Software Entry Tool (Version 3.1) and analyzed using SPSS software Version 15.

# RESULTS

## Results

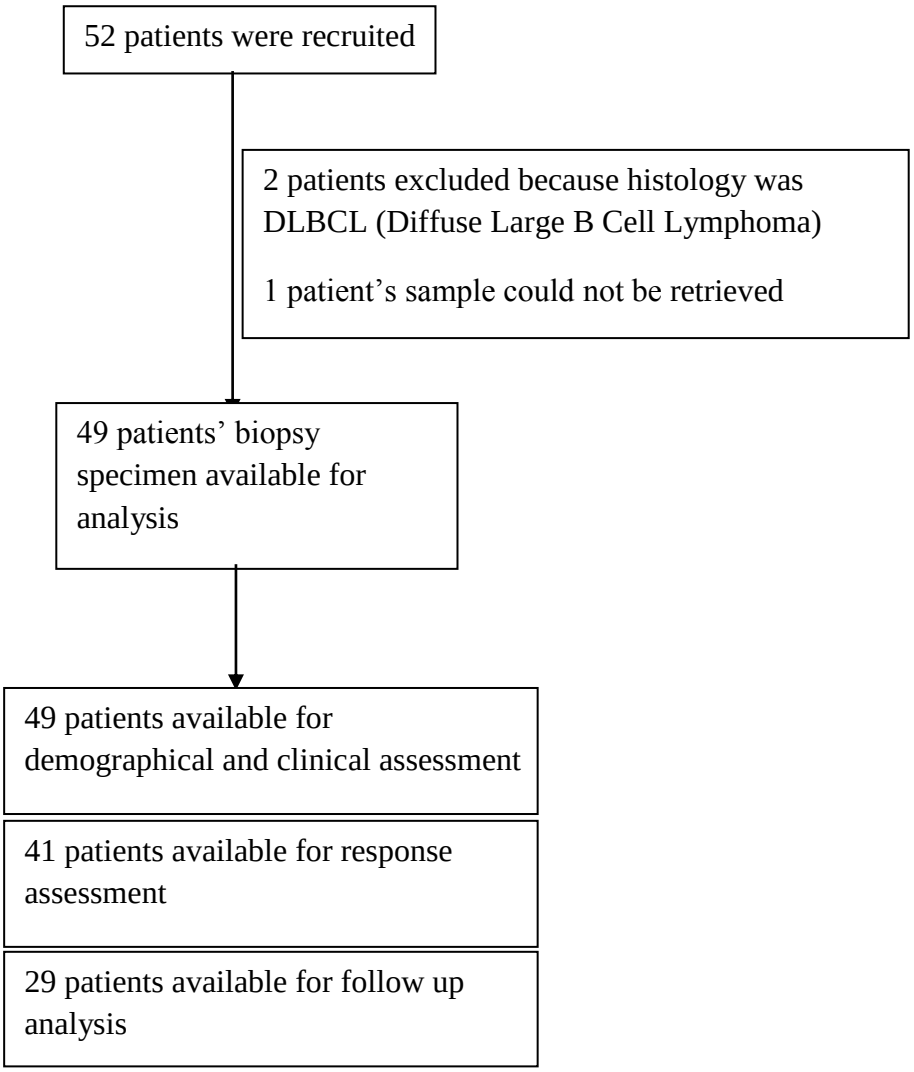

52 patients were recruited
2 patients excluded because histology was DLBCL (Diffuse Large B Cell Lymphoma)
1 patient's sample could not be retrieved
49 patients' biopsy specimen available for analysis
49 patients available for demographical and clinical assessment
41 patients available for response assessment
29 patients available for follow up analysis

Table 6

Demographic and Clinical Variables

| | All Patients (%) |
|---|---|
| Age (in Years) | |
| Median | 56 |
| Range | 25 – 78 |
| TNM Stage | |
| 1 | 5 (10.2) |
| 2 | 5 (10.2) |
| 3 | 13 (26.5) |
| 4 | 26 (53.1) |
| T stage | |
| T1 | 8 (16.3) |
| T2 | 12 (24.5) |
| T3 | 11 (22.4) |
| T4 | 17 (34.7) |
| N stage | |
| N0 | 16 (32.7) |
| N1 | 17 (34.7) |
| N2 | 14 (28.6) |
| | 2 (4.1) |

Table 6 (contd.)

Demographic and Clinical Variables

| | |
|---|---|
| Duration of Symptoms (in months) | |
| Range | 2 – 12 |
| Mean | 3 |
| Management | |
| Surgery +/- Radiotherapy | 25 (51) |
| Radiotherapy +/- Chemotherapy | 16 (32) |
| No Treatment | 8 (17) |
| Tobacco Smoking | |
| Yes | 23 (46.9) |
| No | 26 (53.1) |
| Tobacco Chewing | |
| Yes | 25 (51) |
| No | 24 (49) |
| Alcohol Consumption | |
| Yes | 13 (26.5) |
| No | 36 (73.5) |
| Haemoglobin (n=48) | |
| Range | 9.4 – 15.9 |
| Mean | 13.2 |

Table 6 (contd.)

Demographic and Clinical Variables

| | |
|---|---|
| Site | |
| Oral Cavity | 28 (57) |
| Oropharynx | 20 (41) |
| HIV status | |
| Negative | 48 (98) |
| Positive | 0 (0) |
| Not available | 1 (2) |
| Previous Sexually Transmitted Disease | |
| Yes | 1 (2) |
| No | 48 (98) |
| Previous Anal/Gynaecological Malignancies | |
| Yes | 0 (0) |
| No | 49 (100) |
| Co morbidities | |
| Yes | 23 (46.9) |
| No | 26 (53.1) |

**Figure 7. Sex Distribution**

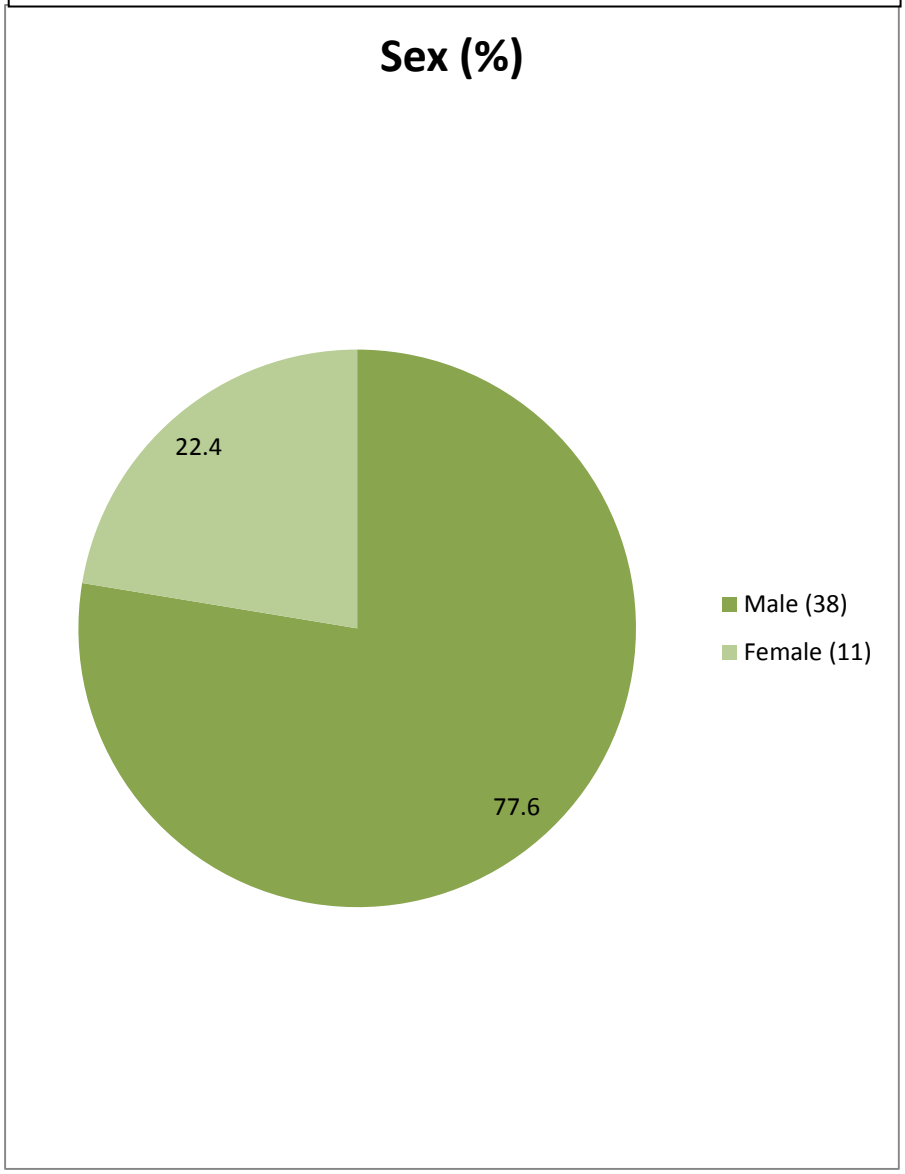
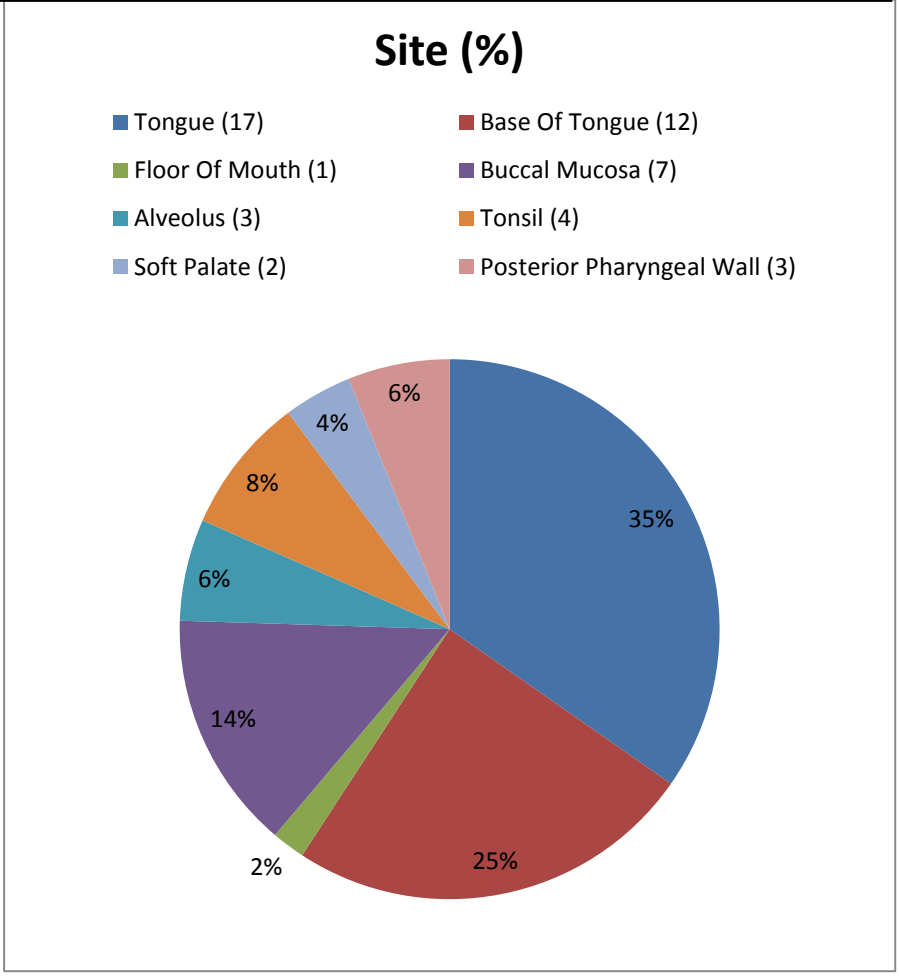


**Figure 8. Site Distribution**




| N = 49 | Oral Cavity (%) | Oropharynx (%) |
|---|---|---|
| Total | 28 (57) | 21 (43) |

**Figure 9. Occupation Distribution**

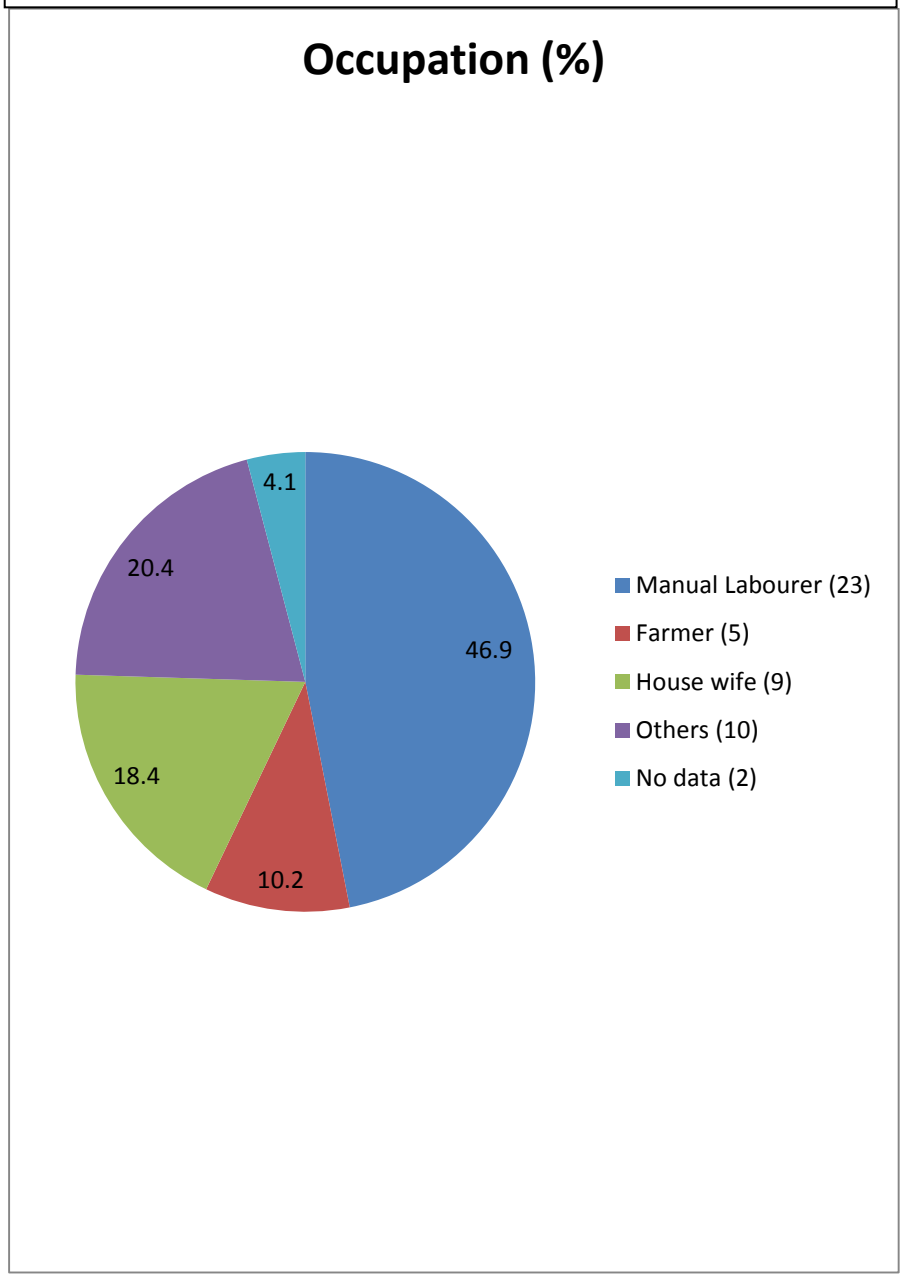


**Figure 10. Performance Status**

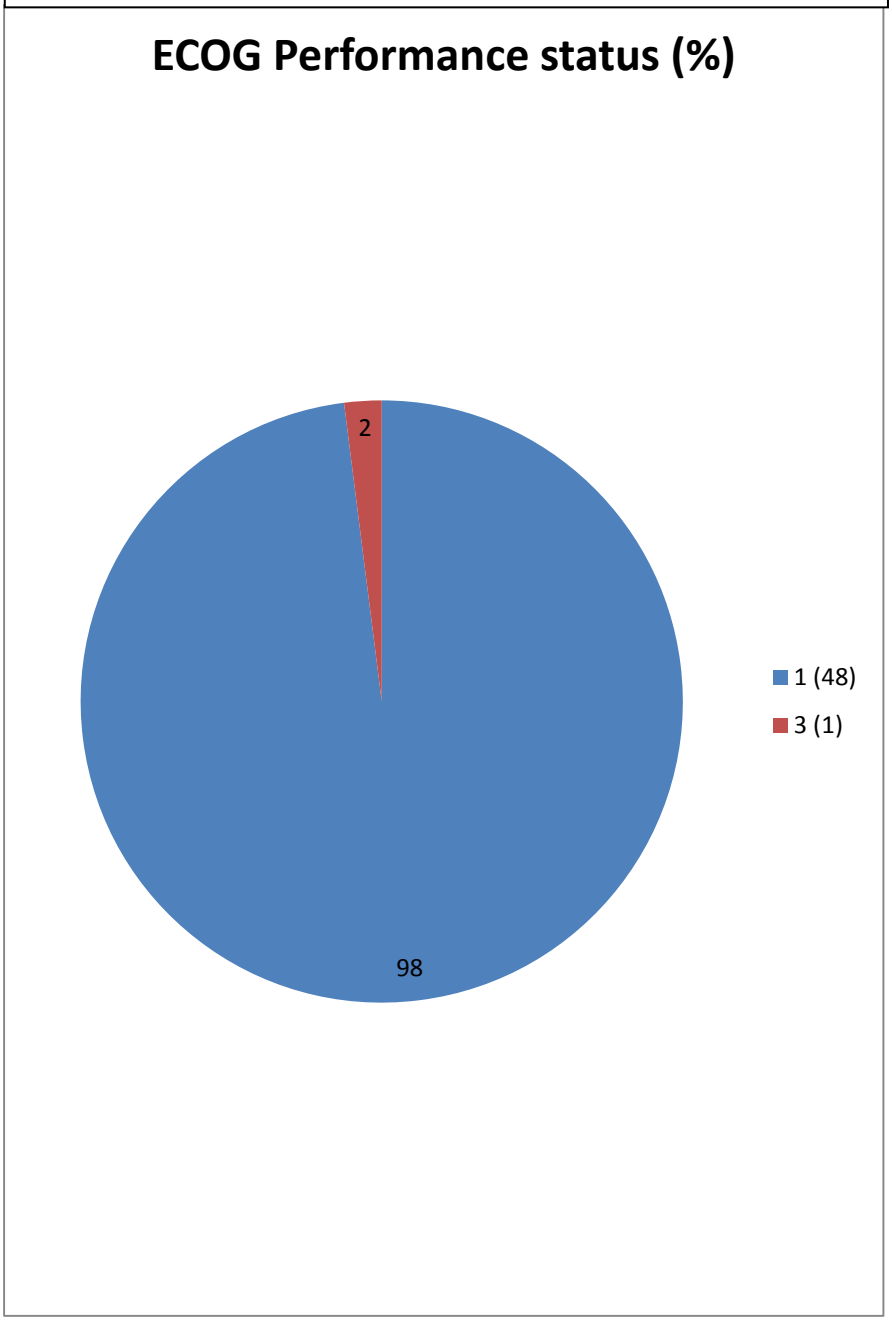


**Figure 11. Education**

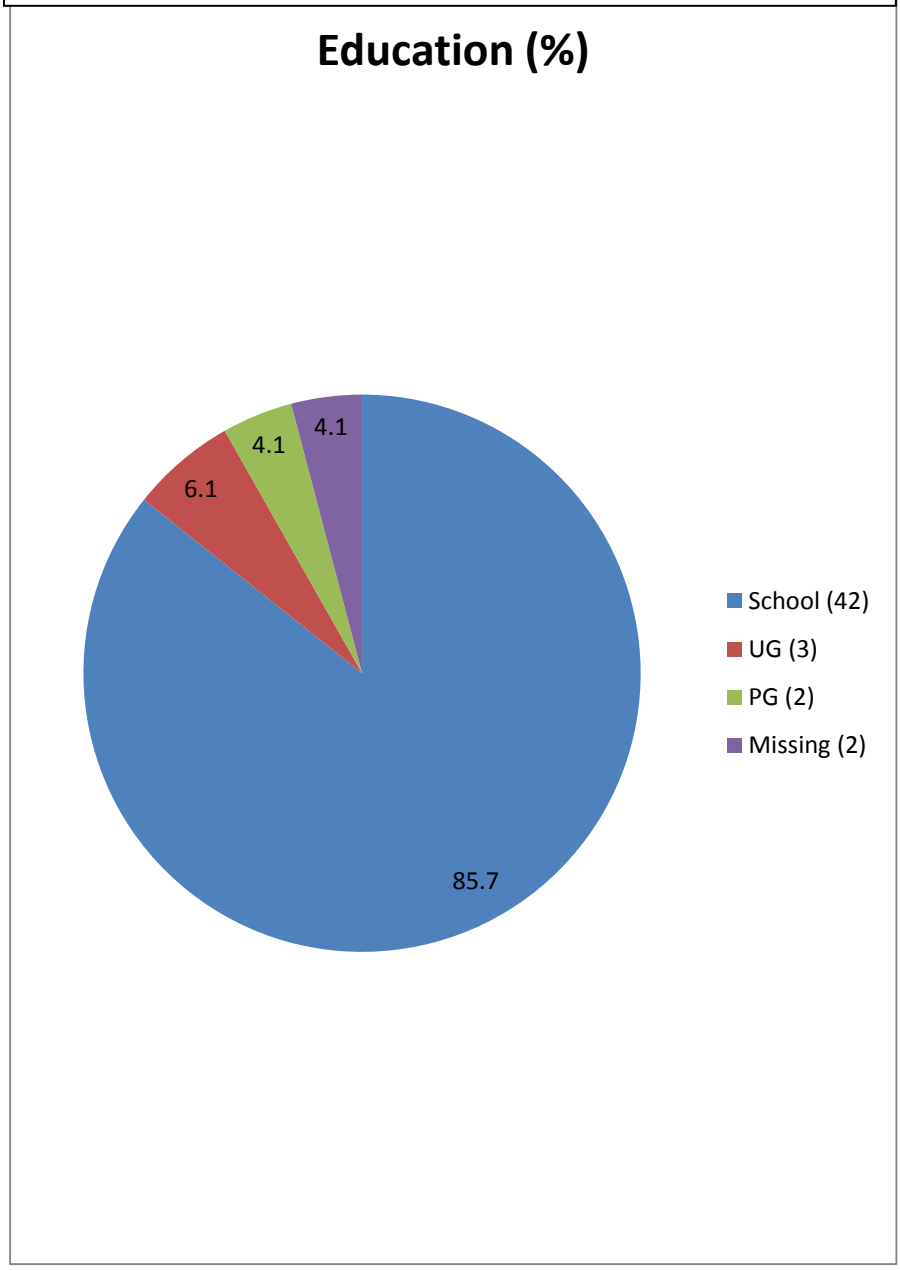


**Figure 12. Pre Malignant Lesions**

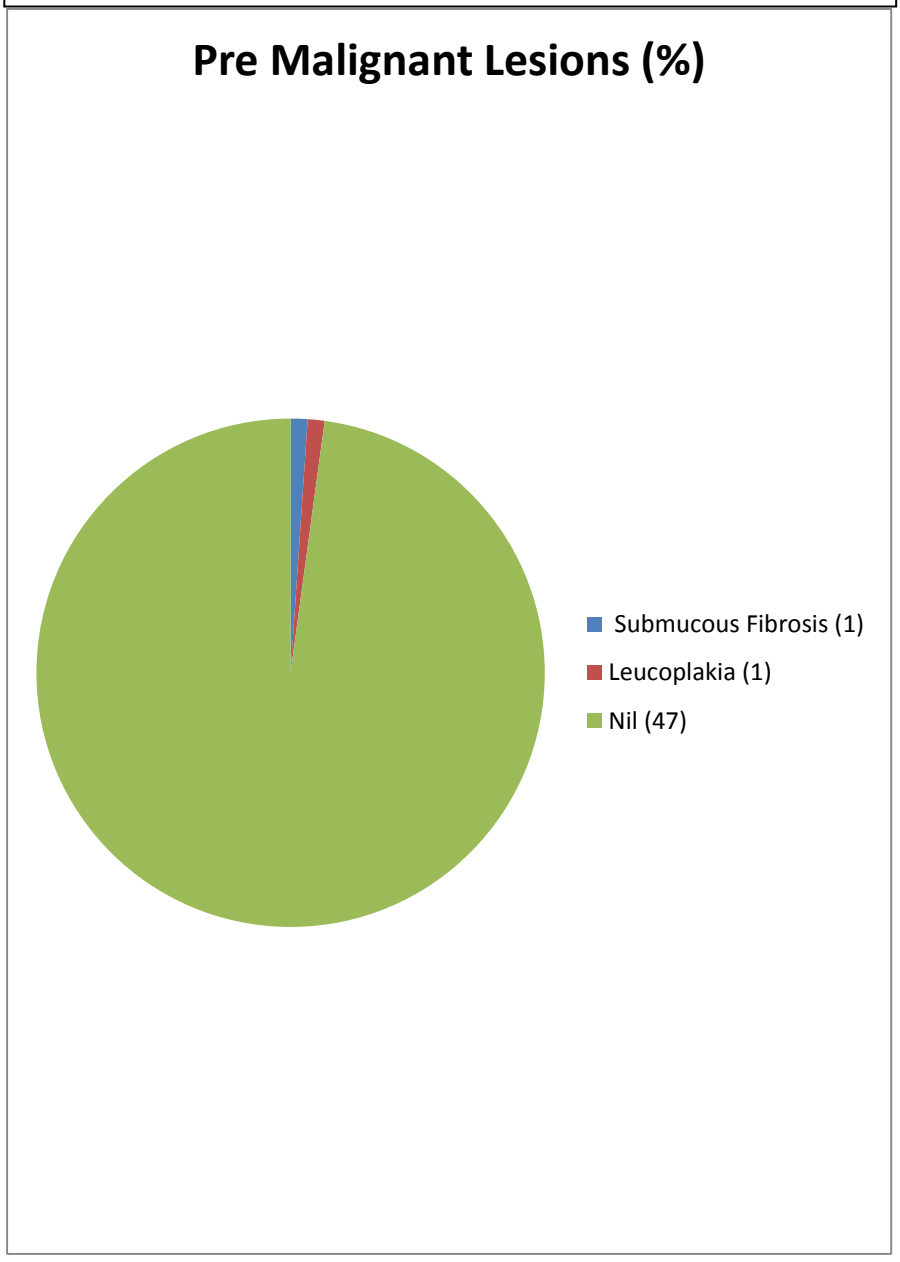


**Figure 13. TNM Stage Distribution**

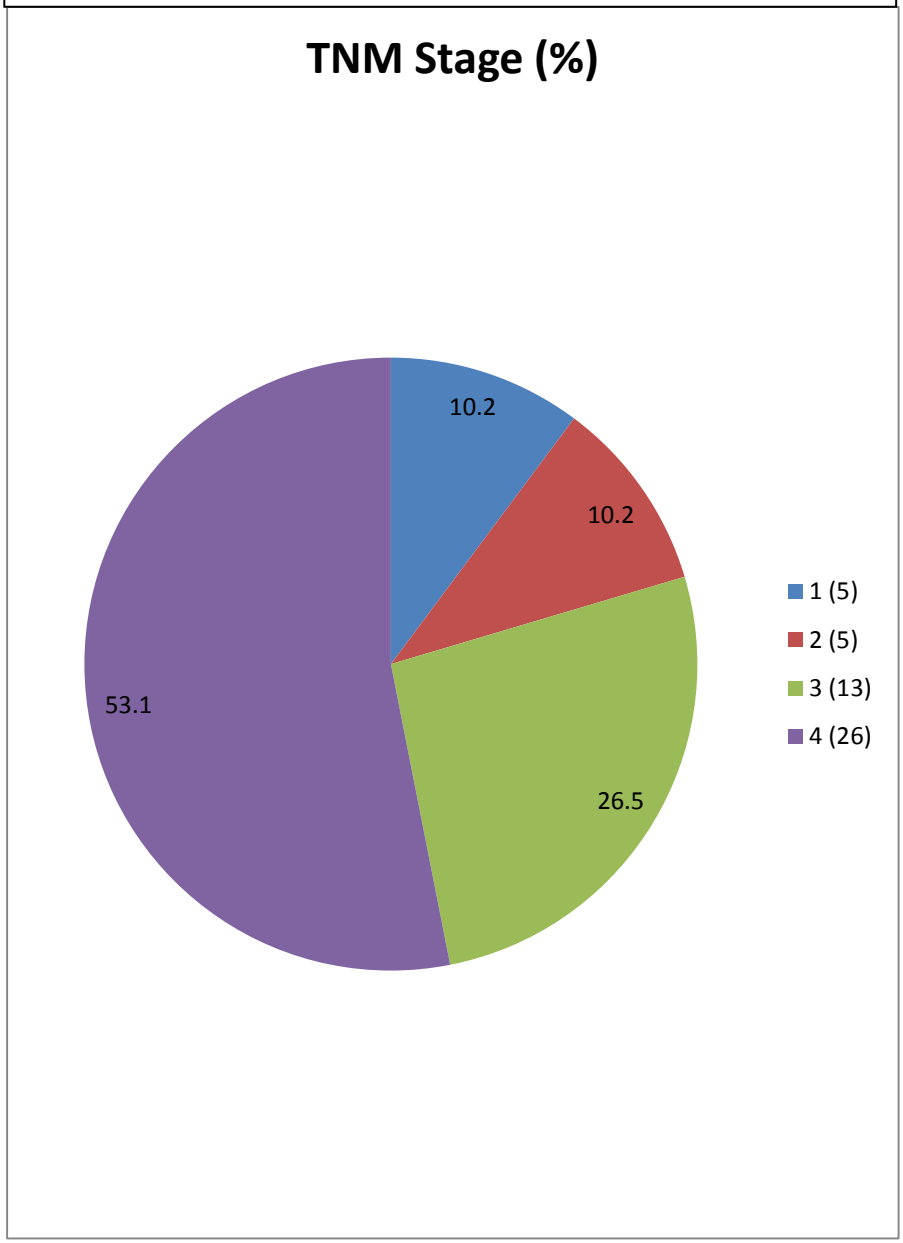
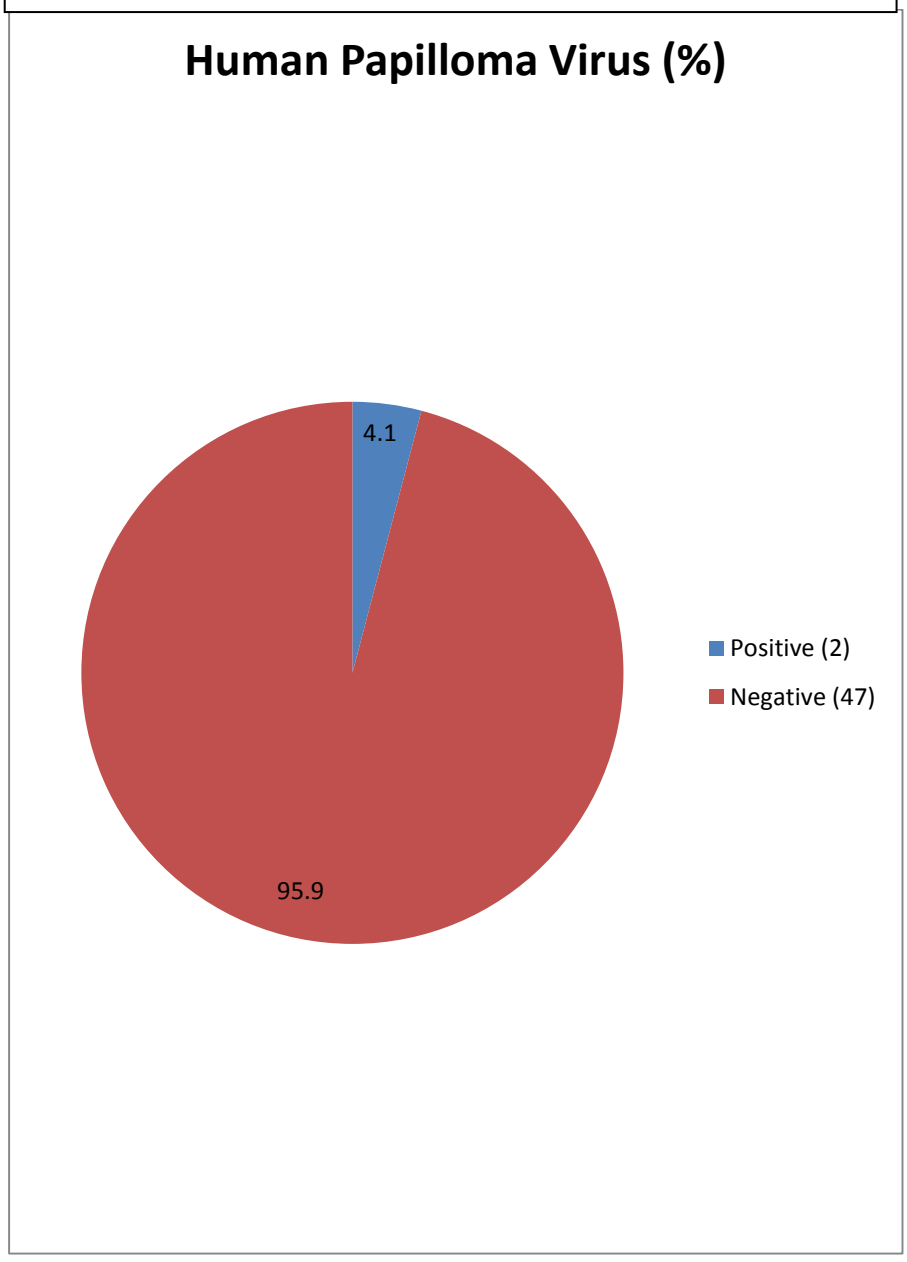


**Figure 14. Human Papilloma Virus (HPV) Distribution**



The median follow up duration was 3 months (Range: 1 – 12 months). The clinical and demographic details are enumerated in Table 6.

The number of patients who underwent surgery as part of their management protocol was higher as compared to radical radiotherapy

There was no difference found between HPV positive and HPV negative patients in terms of age, sex, history of sexually transmitted disease or tobacco/alcohol usage.

**Table 7. Relationship between HPV and Tobacco and Alcohol**

| | HPV positive | HPV negative |
|---|---|---|
| Tobacco/Alcohol present | 2 | 36 |
| Tobacco/Alcohol absent | 0 | 11 |

Performance score was recorded using the ECOG model.(68) It was not analyzed separately as there was only one patient with an ECOG performance score of 3 and all others had a performance score of 1.

There was one HPV positive patient who had associated submucous fibrosis. (Table 8) Otherwise no other relationship could be made out between HPV positive and HPV negative patients. This was not found to be statistically significant.

**Table 8. Relationship between HPV and Pre Malignant Lesions**

| | HPV Positive | HPV Negative |
|---|---|---|
| Sub Mucous Fibrosis | 1 | 0 |
| Leukoplakia | 0 | 1 |
| Nil | 1 | 46 |

Only 29 patients had follow up data recorded. Residual disease at 6 weeks and local recurrence at 3 months was found in 11 patients, of which 1 patient was HPV positive. (Table 9) Regional and Distant Failure was found in 2 of the HPV negative patients and none of the HPV positive patients. This was not found to be statistically significant.

**Table 9. Relationship between HPV and disease outcome**

| | HPV positive | HPV negative |
|---|---|---|
| Local Recurrence/Residual Disease | 1 | 10 |
| Failure (Regional/Distant) | 0 | 2 |
| Disease Free | 1 | 15 |

Only the L1 portion of HPV capsid protein was identified in 2 patients (DNA qPCR). (Figure 12) There was no serotype specific HPV 16 and 18 E6 and E7 gene sequences amplified on RNA qPCR.

A descriptive analysis of these patients has been carried out.

The first patient was a 47 year old male from West Bengal, who was an office worker and had done undergraduate studies. He had duration of symptoms for 7 months. He was a regular tobacco chewer for the past 10 years. He did not use alcohol or smoke cigarettes. He had no history of sexually transmitted disease. He had no family history of malignancy. On evaluation, he was found to have Carcinoma Buccal Mucosa Stage 1 (T1N0M0) with associated sub mucous fibrosis. He had no co morbidities. He underwent surgery (wide local excision + Right Modified Radical Neck Dissection). The post op histopathology report was favourable. He was kept on follow up. He was disease free at last follow up at 6 months.

The second patient was a 56 year old man from Tamil Nadu who was a manual labourer, and had studied till class 10. He had duration of symptoms for 2 months. He was a regular smoker of cigarettes and used to consume alcohol frequently. He did not chew tobacco. He had no history of sexually transmitted disease. He had no family

history of malignancy. He was diagnosed to have Carcinoma Soft Palate Stage III (T3N0M0). He had co morbidities of Diabetes Mellitus on oral hypoglycaemic agents. He underwent radical chemo irradiation. This patient had multiple breaks during radiotherapy (due to personal reasons) and gap correction was done to achieve a tumoricidal dose without the negative effects of stopping treatment midway (accelerated repopulation). He finally received 46 Gy in 23 fractions at 200 cGy/fraction followed by 23.4 Gy in 12 fractions at 195 cGy/fraction, finally achieving a total tumour dose of 69.3 Gy in 35 fractions, with spine shielding at 40 Gy and field size reduction at 49.9 Gy (25 fractions). He also had only 1 cycle of weekly Cisplatin. No further cycles were given as patient had poor tolerance due to grade 3 mucositis and low counts. Assessment at end of treatment revealed residual disease. This was not biopsy proven. He came for follow up after 2 months and then was lost to follow up. Residual disease had increased in size at that time.

# DISCUSSION

## Discussion

In this cohort, there were a large number of patients who used tobacco or alcohol. About 47% of the patients smoked cigarettes, 51% chewed tobacco and 27% of the patients consumed alcohol. When taken together as a group, 78% (n=38) of the patients used one of these substances and 11 patients (22%) had no history of substance abuse.

p16 is known to be associated with good prognosis in HNSCC. (28,69) p53, also known as the guardian of the genome, has a beneficial role to play in the pathogenesis of HNSCC.

It is a known fact that tobacco and alcohol use cause the proposed beneficial effect of being HPV positive to become negated. (58,67) This is because p16 (a cyclin dependant kinase) is down regulated due to several distinct and exclusive events such as homozygous deletion, point mutation, and promoter methylation.(67) p53 mutation also happens in this situation. The combined effect results in decreased responsiveness of the tumour to standard treatment. (58) Thus outcome associated with the HPV positive patients who used tobacco or alcohol would be similar to the HPV negative

group or even worse.(58) Both patients who were HPV positive used some form of tobacco. Concerning response to treatment, one patient had a good response to treatment while the other patient did not have. The reasons for these are not related to HPV but rather to stage and treatment factors. As mentioned in the results section, one patient had early stage cancer and underwent radical surgery He had no local recurrence at last follow up, while the other patient who underwent radical chemo irradiation but had irregular treatment had poor local control. It is well known that squamous cell cancers of the head and neck region are very aggressive tumours and undergo accelerated repopulation if the treatment is not completed within 7-8 weeks, leading to poor local control. (70–72).

One of the patients with oral buccal mucosa cancer with HPV positivity had associated submucous fibrosis (SMF). There is sparse data in the literature about the relationship between HPV and submucous fibrosis.(73–75) Luo et al suggested that oral SMF is associated with low risk HPV serotypes (LR HPV) (other than 16 and 18). Mehrotra et al, in a study done from Allahabad, could not find any correlation between high risk HPV (HR HPV) serotypes and oral SMF. Subsequently Jalouli et al found a HR HPV

prevalence of 91% in oral SMF. But the findings of Jalaouli did not correlate with the above 2 studies. Our patient was found to have HPV positivity (L1 portion of HPV capsid protein was detected), but his E6/E7 RNA gene amplification for HPV 16 and 18 was negative implying he had a serotype other than HPV 16 and 18.

As alluded to earlier, there was scanty number of HPV positive cancers discovered. The reasons for this are multiple and are discussed below.

The total number of oral cancers was more than oropharyngeal cancers. It is known that oropharyngeal cancers have higher association with HPV than other head and neck subsites.(11,76,77) It was decided to add on the other sites as prevalence data had shown HPV positivity to be between 23% - 48% (6,11) in oral subsite as well. This may have contributed to the less number of HPV positive cancers detected. Also, it is well known that HPV positive head and neck cancers are more in the tonsillar subsite of the oropharynx.(27,78,79)The number of tonsillar cancers included

in this study were minimal (8%, n=4), and may have contributed to the low HPV positivity.

The age of the patients were wide spread with most of the patients being more than 50 years (70%). This also goes against the well known fact that most of the patient with HPV positive HNSCC are from the younger age group. (35,80). Hence, this may be a reason for the poor positivity of HPV in our cohort group.

Clinically, HPV positive cancers present with a small primary and large cervical lymph nodes. This was not seen in the 2 patients who were HPV positive. This may be explained by the fact that our patients did not represent the molecular profile of good prognosis HPV cancers because of the presence of tobacco and alcohol, down regulating p16.(58)

Only 1 patient gave history of previous sexually transmitted disease and this patient was not HPV positive. Moreover he was also a smoker and alcohol consumer. It is well known that HPV positive

HNSCC is associated with the sexual route of spread, particularly oral sex. (6,80–82)

Co morbidities were seen in about half of the patients. This is most likely due to the higher age of most of the patients in the cohort. The co morbidities that were recorded were diabetes mellitus, hypertension, coronary artery disease, hypothyroidism and past history of tuberculosis. The second HPV positive patient who did not do well had a co morbidity of being a diabetic. There is some data that suggests that medical co morbidities may play a role in response to treatment.(83,84) Hess et al stated that HPV negative patients have a poorer response to treatment, not only because of their virological status but because HPV negative patients may be more older and would have more co morbidities leading to delays and interruptions of radiation therapy.

# LIMITATIONS

## Limitations

The biggest limitation of this study was the scanty HPV positive patients in the sample pool with more of HPV negative patients. Statistical correlation, therefore, could not be made between the 2 patients who were HPV positive and the 47 who were HPV negative.

The follow up duration was less. Of the 49 patients who were analysed for HPV, only 41 patients had treatment. (8 patients left the institution without treatment) Of the 41 patients who underwent treatment, only 29 patients had any follow up, with a median follow up period of 3 months.

In this study, only fresh tissue was used for HPV detection, and so needed a collection of sample from the patient even before histological diagnosis was confirmed. This also meant that consent for the same was taken before the patient had a confirmed diagnosis of cancer.

The history of oral sex or multiple sex partners were not asked for in our cohort of patients. It is well known that HPV associated HNSCC is associated with oral sex/multiple sexual partners. (6,81,82). We tried to use a surrogate of the same, asking for a history of sexually transmitted disease, looking at HIV status, and asking for a history of previous anal or gynaecological malignancies (in particular carcinoma cervix). This was because of the sensitive nature of the questions that needed to be asked. Most western data where this association was proved used a self administered questionnaire to the patient and the data was anonymously collected thus facilitating the process. It also may have led to more accurate data being collected as patients may not reveal the correct nature of their activities when being asked for the same by an investigator filling a proforma.

It would have been appropriate to look at other molecular markers that have shown good association with HPV positive HNSCC such as p16 and p53. (58) But this was not possible due to technical reasons.

# CONCLUSION

## Conclusions

The survival assessment between HPV positive and negative cancers could not be made out because of the small number of HPV positive cancers as well as the short duration of follow up.

Future research probably should look at only the oropharynx and tongue subsites in search of HPV positive cancers as these are the subsites which have shown high positivity in the Indian context. Also, in addition, the buccal mucosa subsite would be a worthy addition to the sites harbouring HPV (one of our patients who was HPV positive had a buccal mucosa primary).

The association of oral submucous fibrosis with HPV was a chance finding in our study. This patient was also a regular tobacco chewer. This was the more probable reason for him to develop submucous fibrosis. Very sparse data is available on the association of HPV with submucous fibrosis in the literature. Future research needs to look at the association of HPV with oral pre malignant conditions like oral submucous fibrosis as well as other lesions such as

leukoplakia and erythroplakia, all of which have traditionally been associated with tobacco and betel nut chewing.

Among our patient cohort 78% were tobacco/alcohol users and this could probable be one of the reasons for the low rates of HPV detection in our study population.

There is a need to look at the molecular profile like p16 and p53 in these patients as that is more significantly associated with prognosis.

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# APPENDIX

# Appendix

## ECOG Performance Score

| Grade | ECOG |
|---|---|
| 0 | Fully active, able to carry on all pre disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature |
| 2 | Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self care. Totally confined to bed or chair |
| 5 | Dead |

# Spread Sheet Of Data

| ser | name | hos no | rt no | age | sex | addres | ph no |
|---|---|---|---|---|---|---|---|
| 1/13 | Gulzar Khan | 410300f | 281/13/rt2 | 55 | 1 | Vellore | 82206831 |
| 2/13 | Riyaz Ahmed | 397561b | 49/13/rt2 | 48 | 1 | Vellore | 822066750 |
| 3/13 | Bojraj | 398209f | nil | 41 | 1 | Chennai | 979075430 |
| 4/13 | Munniswamy | 417419f | 208/13/rt2 | 60 | 1 | Vellore | 915916007 |
| 7/13 | Donapati Gosh | 414758f | 245/13/rt2 | 50 | 1 | West Bengal | 947597989 |
| 5/13 | Sankar V | 701163b | 292/13/rt2 | 31 | 1 | Vellore | 989454433 |
| 8/13 | Sapan Dey | 268593f | 278/13/rt2 | 47 | 1 | West Bengal | 905121688 |
| 9/13 | Iman Ali | 432094f | 283/13/rt2 | 64 | 1 | Assam | 995406186 |
| 10/13 | A. Kuppam | 493729f | 616/13/rt2 | 59 | 1 | Thiruvanamalai | 994499372 |
| 11/13 | Bhuvanasundaram | 491760f | 545/13/rt2 | 63 | 1 | Kuppam | 994076167 |
| 12/13 | Atma Ram Sharma | 499374f | 578/13/rt2 | 76 | 1 | West Bengal | 983072376 |
| 13/13 | Subramani | 275766f | 623/13/rt2 | 64 | 1 | Vellore | |
| 14/13 | Dilip Kaur | 494718f | | 59 | 2 | Jharkhand | 776068411 |
| 15/13 | Nitai Hait | 608479f | 672/13/rt2 | 57 | 1 | West Bengal | 973281649 |
| 16/13 | Anisur Sahaji | 600354f | 653/13/rt2 | 25 | 1 | West Bengal | 912606063 |
| 17/13 | Jayavathi Jesudoss | 475707f | 666/13/rt1 | 75 | 2 | Tirupathur | 948933564 |
| 18/13 | Moidu K.K. | 488070f | 675/13/rt2 | 54 | 1 | Kerala | 904881732 |
| 19/13 | Amiya kumar Sinha | 648497f | 762/13/rt2 | 63 | 1 | West Bengal | 990332360 |
| 20/13 | Md Rukomazammen | 645133f | 756/13/rt2 | 49 | 1 | Bangladesh | 97862563 |
| 21/13 | Niranjan Debnath | 668369f | nil | 47 | 1 | West Bengal | 986243708 |
| 22/13 | Probir Mondal | 670816f | nil | 47 | 1 | West Bengal | |
| 23/13 | Ali Hassan | 706215f | 58/14/r2 | 75 | 1 | Kerala | 994617008 |
| 24/13 | Mehoronessa | 723492f | 28/14/rt1 | 63 | 2 | Bangladesh | |
| 25/13 | bhagyeshwar Patnagia | 729689f | 1038/13/rt2 | 54 | 1 | Assam | 970755673 |
| 26/13 | benedith azuka | 776721f | 39/14/rt2 | 60 | 2 | nigeria | 915909320 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 27/13 | Venkatesan a. s. | 714758f | 158/14/rt2 | 62 | 1 | Vellore | |
| 28/13 | Mosammet | 770191f | 104/14/rt2 | 62 | 2 | Bangladesh | 171861790 |
| 29/13 | Gita Haran Sah | 789934f | 132/14/rt2 | 63 | 1 | West bengal | 193216957 |
| 30/13 | Selva nalathambi | 727627f | nil | 48 | 1 | Ranipet | 989415962 |
| 31/13 | Ramamurthy R. | 793849f | 31/13 | 56 | 1 | Vellore | 909211719 |
| 32/13 | Anil Chandra | 794989f | nil | 64 | 1 | Bangladesh | 960093792 |
| 33/13 | maadhu | 778553f | nil | 40 | 1 | Vellore | 995239917 |
| 34/14 | Ishwer Kumar Singh | 796563f | Nil | 58 | 1 | Jharkhand | 993410886 |
| 35/13 | Apgani Begum | 789601f | 309/14/rt1 | 61 | 2 | Assam | 985442799 |
| 36/13 | Jose VK | 776863f | nil | 55 | 1 | Kerala | 964565407 |
| 37/13 | Sarita Agarwal | 790887f | Nil | 47 | 2 | West Bengal | 943410165 |
| 38/13 | Bhipad Banjan | 828094f | Nil | 65 | 1 | West bengal | 964718116 |
| 39/13 | Saraswati Pandit | 832213f | Nil | 78 | 2 | West bengal | 98745303 |
| 40/13 | Madhava Chakraborty | 834803f | 469/14/rt1 | 46 | 1 | Bangladesh | 97466581 |
| 41/13 | Rekha Aktar | 806099f | 375/14/rt1 | 49 | 2 | Bangladesh | 9.1842E+1 |
| 42/13 | Anjan Kumar Das | 801865f | 460/14/rt2 | 54 | 1 | West Bengal | 943447144 |
| 45/13 | mantu Roy | 803572f | 403/14/rt1 | 48 | 1 | West Bengal | 983050502 |
| 46/13 | Natesan V | 502676d | 580/14/rt2 | 70 | 1 | Tiruvanamalai | |
| 47/13 | Basubi Mondal | 847120f | 610/14/rt2 | 54 | 2 | West Bengal | 974864090 |
| 49/13 | Johnson P. George | 870018f | 593/14/rt1 | 52 | 1 | Kerala | 94956655 |
| 50/13 | Saraswathy R. | 865328f | 609/14/rt2 | 60 | 2 | Coimbatore | 994434005 |
| 51/13 | Sirajul Haque | 867702f | nil | 56 | 1 | Bangladesh | |
| 52/13 | Naseem Ahmed | 008846g | Nil | 54 | 1 | West Bengal | 933346291 |
| 53/13 | Mithu Chandra | 001468g | nil | 50 | 1 | West Bengal | 947487077 |

| occupation | educat | dur symp | smoking | smokeless tobacco | alcohol | pre malig | co morb | sex tr dis |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 1 | 1 | 1 | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE |

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| 2 | 1 | 9 | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 1 | 1 | 3 | FALSE | TRUE | FALSE | FALSE | TRUE | FALSE |
| 1 | 1 | 9 | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 2 | 1 | 6 | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 2 | 1 | 3 | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE |
| 1 | 1 | 2 | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE |
| 4 | 1 | 4 | TRUE | TRUE | FALSE | FALSE | TRUE | FALSE |
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| 2 | 1 | 7 | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 3 | 1 | 3 | FALSE | TRUE | FALSE | FALSE | TRUE | FALSE |
| 1 | 1 | 4 | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 1 | 2 | 6 | TRUE | TRUE | TRUE | FALSE | TRUE | FALSE |
| 4 | 1 | 2 | TRUE | TRUE | FALSE | FALSE | TRUE | FALSE |
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| 1 | 1 | 12 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
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| 1 | 1 | 6 | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE |
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| 4 | 2 | 6 | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE |
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| 1 | 1 | 3 | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 3 | 1 | 3 | FALSE | TRUE | FALSE | FALSE | TRUE | FALSE |
| 4 | 2 | 2 | FALSE | TRUE | TRUE | FALSE | TRUE | FALSE |
| 3 | 1 | 3 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| | | 3 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
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| 4 | 1 | 3 | TRUE | FALSE | TRUE | FALSE | TRUE | FALSE |
| 3 | 1 | 3 | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 1 | 1 | 6 | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 1 | 1 | 6 | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 1 | 1 | 2 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |

| pr malig | PS | site | stage | t | n | hb | cr | |
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| FALSE | 1 | 1 | 3 | | 3 | 1 | 14.4 | 1.36 |
| FALSE | 1 | 4 | 4b | | 4 | 1 | 12.3 | 1.15 |

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|---|---|---|---|---|---|---|---|
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| FALSE | 1 | 2 | 4a | 3 | 2 | 14.1 | 0.84 |
| FALSE | 1 | 8 | 4b | 4 | 1 | 11.9 | 1.03 |
| FALSE | 1 | 6 | 3 | 3 | 0 | 12.2 | 1.39 |
| FALSE | 1 | 2 | 4a | 4 | 2 | 14.2 | 0.83 |
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| FALSE | 1 | 1 | 2 | 2 | 0 | 9.4 | 3.06 |
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| surgery | rt | ch rt | rt breaks | hpv | e6 e7 | p16 | residual |
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| FALSE | TRUE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| TRUE | FALSE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| FALSE | TRUE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| FALSE | FALSE | TRUE | FALSE | FALSE | 0 | FALSE | FALSE |
| FALSE | TRUE | FALSE | TRUE | FALSE | 0 | FALSE | FALSE |
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| TRUE | TRUE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| TRUE | TRUE | FALSE | TRUE | FALSE | 0 | FALSE | FALSE |
| TRUE | FALSE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| TRUE | FALSE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |
| TRUE | FALSE | FALSE | FALSE | FALSE | 0 | FALSE | FALSE |

| ti loc | ti reg | ti met | ti death | tot fol up |
|---|---|---|---|---|
| 3 | 1 | 3 | 3 | 3 |
| 3 | 6 | 6 | 6 | 6 |
| | | | | 0 |
| 3 | 3 | 6 | 6 | 6 |
| 3 | 4 | 4 | 4 | 4 |
| 3 | 6 | 6 | 6 | 6 |
| | | | | 0 |
| 12 | 12 | 12 | 12 | 12 |
| | | | | 0 |
| 6 | 6 | 6 | 6 | 6 |
| 3 | 3 | 3 | 3 | 3 |
| 9 | 9 | 9 | 9 | 9 |
| 12 | 12 | 12 | 12 | 12 |
| | | | | 0 |
| 3 | 4 | 4 | 4 | 4 |
| 9 | 9 | 9 | 9 | 9 |
| 12 | 12 | 12 | 12 | 12 |

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|---|---|---|---|---|
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| | | | | 0 |
| 6 | 6 | 6 | 6 | 6 |
| 9 | 9 | 9 | 9 | 9 |
| 3 | 3 | 3 | 3 | 3 |
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| | | | | 0 |
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| 3 | 3 | 3 | 3 | 3 |
| 3 | 3 | 3 | 3 | 3 |
| | | | | 0 |
| | | | | 0 |
| **2** | 3 | 3 | 3 | 3 |
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| 3 | 3 | 3 | 3 | 3 |
| | | | | 0 |
| 4 | 3 | 4 | 4 | 4 |
| 6 | 6 | 6 | 6 | 6 |
| 3 | 3 | 3 | 3 | 3 |
| | | | | 0 |
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| 1 | 1 | 1 | 1 | 1 |
| 3 | 3 | 3 | 3 | 3 |
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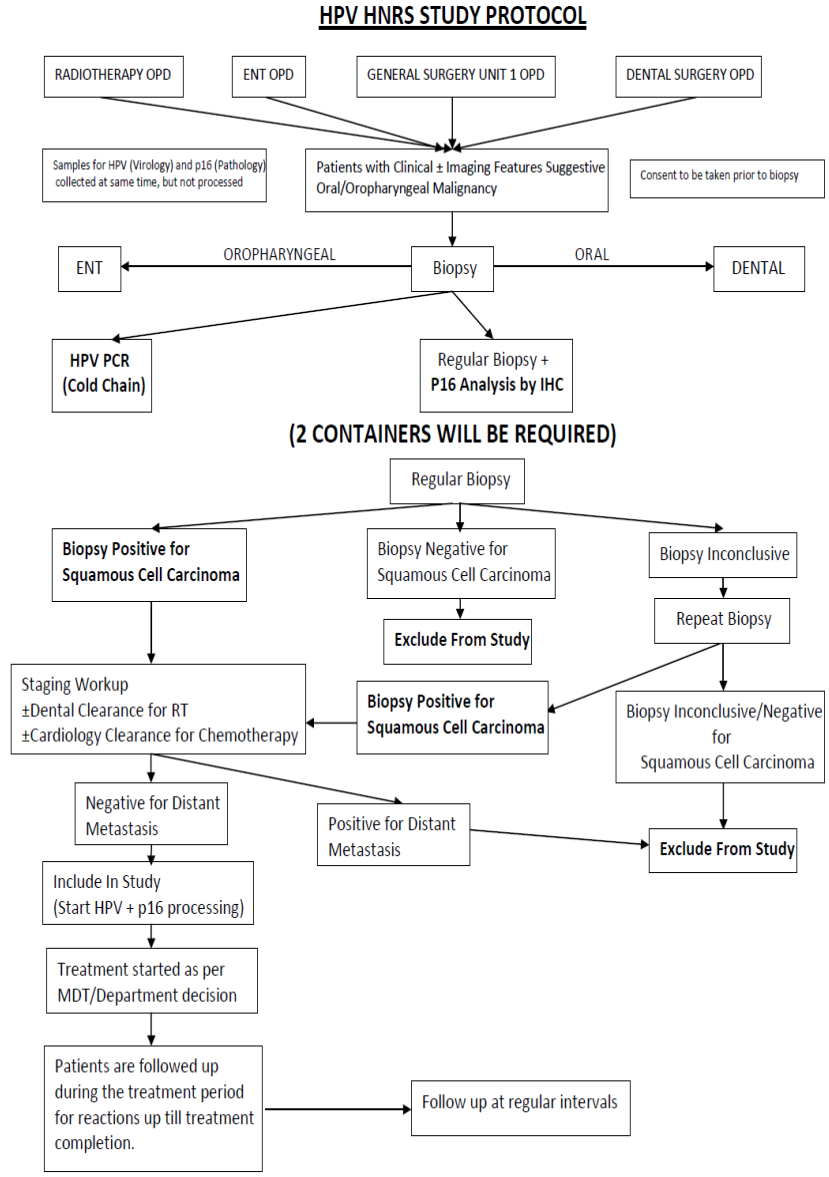
# Protocol Flow Chart



## Study Proforma

**PATIENT ID:**

Name: Hosp.No. R.T. No.

Age : DOB: Sex:

Address: .

Local Permanent

Ph no Ph no

Marital Status

Socioeconomic status: 1. Patient's Occupation:

2. Patient's Education :

Presenting complaints:

| | Duration |
|---|---|
| Throat pain | Y/N |
| Dysphagia(liquids/semisolids/solids) | Y/N |
| Ulcer | Y/N |
| Bleeding | Y/N |
| Hoarseness | Y/N |
| Cough | Y/N |

Stridor Y/N

Neck swelling: Y/N

Headache: Y/N

Trismus: Y/N

Ear Ache: Y/N

Others:

Addictions:

1. Smoking Y/N Packs per day- Duration - /yrs

2. Smokeless Tobacco Y/N Duration - /yrs

3. Alcohol Y/N Amount & frequency - Duration - /yrs

Associated diseases: Premalignant conditions

Leukoplakia

Erythroplakia

Submucous Fibrosis

Dysplasia

DM/ HT/ TB/ Others

Allergies

Sexually Transmitted Diseases

Past history: Previous Oral/Anal/Gynaecological malignancies

GENERAL EXAMINATION:

Performance status: ECOG

Physical parameters :Weight- Height- BSA-

Vitals :BP- PR-

Pallor/ Icterus/ Edema /

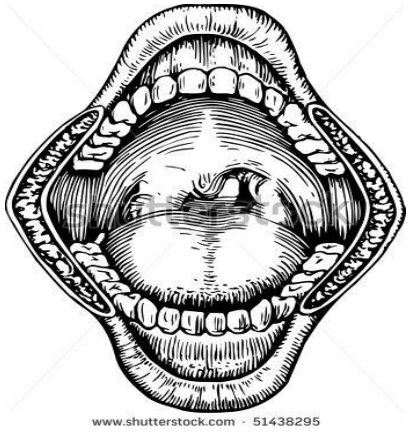
Tracheostomy / Ryle's tube

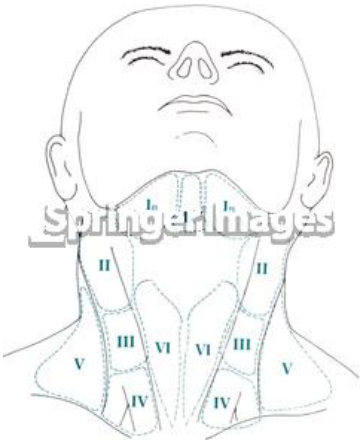
Systemic examination: CVS

RS

Abdomen

<u>Local examination</u>



Oral Cavity

| | Lip | Buccal Mucosa | Tongue | Upper Alveolus | Lower Alveolus | Hard Palate | Floor of Mouth |
|---|---|---|---|---|---|---|---|
| Size | | | | | | | |
| Nature | | | | | | | |
| Others | | | | | | | |

Oropharynx

| | Base Of Tongue | Tonsil | Posterior pharyngeal wall | Uvula and Soft Palate |
|---|---|---|---|---|
| Size | | | | |
| Nature | | | | |
| Others | | | | |

NPL scopy /IDL

**Neck-**

Lymph nodes-Y/N

| Side | Level | no | Size | Mobile/ Fixed | Discrete/ Matted | Skin –Free/ Tethered Ulcerated |
|---|---|---|---|---|---|---|
| right | 1 | | | | | |
| | 2 | | | | | |
| | 3 | | | | | |
| | 4 | | | | | |
| | 5 | | | | | |
| left | 1 | | | | | |
| | 2 | | | | | |
| | 3 | | | | | |
| | 4 | | | | | |
| | 5 | | | | | |

Clinical Diagnosis:

**Site:** **T N M** **Stage:**

**INVESTIGATIONS**

Haemogram (date: ) HB- TC- DC -
Platelets-

Biochemistry (date: ) Creatinine-
Creatinine clearance –

LFT-

Virology (date: ) HIV- HCV- HbsAg-

Chest xray (date: ) -

CT scan (date: ) -

Histopathology Biopsy No ( / )-

(date: ) Squamous cell carcinoma

FNAC: Site - Report -

(date: )

Post op Histopathology Biopsy No ( / )-

(date: ) Squamous cell carcinoma

Well / moderately / poorly differentiated

Size

Margins –

Lymph nodes: No-

extracapsular invasion: Y/N

Lymphatic / vascular invasion - Y/N

p

**Final diagnosis**

Site T N M Stage

Sub Site

Any other details:

**TREATMENT DETAILS:**

Primary Radical Surgery followed by Post Op RT
Primary Radical Surgery followed by Post Op Chemoirradiation
Primary Radical Radiotherapy
Primary Radical Radiotherapy followed by Salvage Surgery
Primary Radical Radiotherapy with concurrent chemotherapy followed by Salvage Surgery
Primary Radical Radiotherapy with concurrent biological therapy
Palliative Radiotherapy

Overall treatment time:

Date started:

Date completed:

Machine: Co 60 / 6X

**Dose**

Fractions / week:

Technique:

TD / #

**Treatment Break** Yes/No

| No of breaks | Duration in days | Reason |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |

HPV STATUS

Genotype 16/18 –

E6/E7 RNA quantification: copies/ml

p16 STATUS

Positive / Negative

# DETAILED FOLLOW UP PLAN

**1ST FOLLOW UP AT 6 WEEKS**:

1. HISTORY AND PHYSICAL EXAMINATION

2. NPL SCOPY (OROPHARYNGEAL)

3. POST RT ASSESSMENT

**2ND FOLLOW UP AT 3 MONTHS**:

1. HISTORY AND PHYSICAL EXAMINATION

2. NPL SCOPY (OROPHARYNGEAL)

3. POST RT ASSESSMENT

**3RD FOLLOW UP AT 6 MONTHS**:

1. HISTORY AND PHYSICAL EXAMINATION

2. NPL SCOPY (OROPHARYNGEAL)

3. CHEST XRAY

**4TH FOLLOW UP AT 9 MONTHS:**

1. HISTORY AND PHYSICAL EXAMINATION

2. NPL SCOPY (OROPHARYNGEAL)

**5TH FOLLOW UP AT 12 MONTHS:**

1. HISTORY AND PHYSICAL EXAMINATION

2. NPL SCOPY (OROPHARYNGEAL)

3. CHEST XRAY

**Informed Consent**

# Christian Medical College, Vellore

## Department of Radiotherapy Unit 2

## Disease Outcome in Oral and Oropharyngeal Squamous Cell Cancers based on Combined Virological and Molecular Risk Stratification. (HPV HNRS STUDY)

## ***Patient Information sheet***

You are being asked to take part in a study to find out whether outcome of your disease is affected by certain additional tests (HPV and p16) which will be done on the mandatory diagnostic biopsy.

**If you decide to take part, what will you have to do?**

You would be asked to answer a few questions about yourself, your habits and other personal details. Also, in addition to the biopsy taken to confirm the disease, an additional small amount of tissue would be sent for special testing. There is no need to worry about this because this tissue would be removed at the time of the diagnostic biopsy. There would be no additional cost to you, except that which you would otherwise pay for a normal biopsy. Once your treatment is completed, you would be expected to come for regular checkups to check on your disease status. The additional tests would be performed only after the diagnostic biopsy report is ready. If the diagnostic biopsy is inconclusive for cancer, no special tests would be done and you would be informed of the same. You would also be asked to undergo a repeat biopsy at that time to confirm the disease.

There is a chance that the biopsy report shows a rare type of cancer which is not associated with the additional tests that are performed. In that case also, the tests would not be done and you would be informed of the same.

**Can you withdraw from this study after it starts?**

Your participation in this study is entirely voluntary and you are free to decide to withdraw permission to participate in this study. If you decide to do so, this would not affect your usual treatment at this hospital in any way. .

**Will you have to pay for the extra tests?**

As explained above, you would have to pay for the mandatory diagnostic biopsy prior to the diagnosis of cancer, but the extra tests would be done without any cost to you.

**What happens after the study is over?**

The results of this study may not benefit you directly but it would definitely help to design better treatments for people with similar problems in the future.

**Will your personal details be kept confidential?**

The results of this study may be published in a medical journal but you would not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, if you decide to participate in this study.

**If you have any further questions, please ask**

**Dr. Andrew C. Fenn**

**Dept Of Radiotherapy Unit 2**

**CMC, VELLORE**

**andrew_fenn@rediffmail.com**

**CONSENT TO TAKE PART IN A CLINICAL TRIAL**

**Study Title**: ***Disease Outcome in Oral and Oropharyngeal Squamous Cell Cancers based on Combined Virological and Molecular Risk Stratification (HPV HNRS)***

**Study Number:**

**Participant's name:**

**Date of Birth / Age (in years)**:

I_____
_____,

son/daughter/wife of
_____

(Please tick boxes)

**Declare that I have read the information sheet provide to me regarding this study and have clarified any doubts that I had. [ ]**

**I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]**

**I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]**

**I understand that my identity will not be revealed in any information released to third parties or published [ ]**

**I voluntarily agree to take part in this study [ ]**

Name:

Signature/ Thumb Impression:

Date:

Name of witness:

Relation to participant:

Date:

## Glossary of Terms

HPV – Human Papilloma Virus

LR HPV – Low Risk Human Papilloma Virus

HR HPV – High Risk Human Papilloma Virus

HR HPV ISH – High Risk Human Papilloma Virus In Situ Hybridisation

p16 – D-type cyclin-dependent kinase

ENT – Ear, Nose and Throat

RT – Radiotherapy

DNA – Deoxy Ribonucleic Acid

RNA – Ribonucleic Acid

PCR – Polymerase Chain Reaction

HNSCC – Head and Neck Squamous Cell Cancers

RCT – Randomized Control Trial

NPL Scopy – Naso Pharyngo Layngoscopy

TNM – Tumour Node Metastasis

ECOG – Eastern Co-operative Oncology Group